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# ALLEN'S COMMERCIAL ORGANIC ANALYSIS

FIFTH EDITION, REWRITTEN, REVISED, RESET  
COMPLETE IN TEN VOLUMES EACH VOLUME SOLD SEPARATELY

The organic chemicals and products employed in the arts, manufactures, commerce, medicine, science, etc. It treats upon the properties, modes of analysis, proximate analytical examination; methods for detection and estimation of impurities, adulterations, products of decomposition, etc.

## CONTENTS OF VOLUME I

**Introduction.** By W. A. DAVIS, B. Sc., A. C. G. I., Rock Ferry, Cheshire, Eng. Preliminary Examination; Specific Gravity; Changes in Physical State; Optical Properties; Spectrometers and Spectrographs; Polarimeters; Arrangements for Maintaining a Known Constant Temperature; Ultimate Analysis; Moisture, Crude Fibre and Ash; Action of Solvents.

**Alcohols.** By L. M. BURGHART, M. A., Baltimore, Md. Methyl Alcohol; Wood Naptha; Crude Wood Spirit; Acetone; Ethyl Alcohol; Higher Aliphatic Alcohols.

**Malt and Malt Liquors.** By JULIAN L. BAKER, F. I. C., Staines, Eng. Malt; Malt Worts; Roasted Barley and Malt; Brown and Crystal Malts; Malt Substitutes; Grits and Raw Grain; Malt Extract; Caramel; Invert Sugar; Starch Sugars; Preparation of Materials; Beer and Ale.

**Wines and Potable Spirits.** By LEWIS EYNON, B. Sc., F. I. C., London. Wines; Significance of Results of Wine Analysis; Cider; Potable Spirits.

**Yeast.** By EMIL SCHLICHTING, PH. D., New York. Yeast; Culture Yeast; Pure Culture of Yeast and Its Application in Practice; Physical Examination of Yeast.

**Neutral Alcohol Derivatives.** By HENRY LEFFMANN, M. D., PH. D., Philadelphia. Ether; Aldehydes; Method of Determining Chloroform in Medicinal Preparations.

**Sugars.** By LEWIS EYNON, B. Sc., F. I. C., London. Classification; Methods of Analysis Depending on Specific Gravity or Solution Density; Methods of Analysis Depending on Optical Activity; Method of Analysis Depending on Refractive Index; Methods of Analysis Depending on Reducing Power; Method of Analysis Depending on Oxidation with Iodine; Methods of Analysis Depending on Fermentation; Cane Sugar, Analysis and Valuation of Cane and Beet Sugar Products; Sucrose in Beetroot; Maltose; Lactose; Monosaccharides; Honey; Maple Products; Urine Analysis; Pentoses.

**Starch and Its Isomerides.** By T. H. POPE, B. Sc., F. I. C., Wallasey, Cheshire, Eng. Starch; Estimation of Starch; Dextrine, Amylin; Cellulose; Gums;

Proximate Analysis of Plants; Cereals; Wheat, Flour; Bread; Macaroni; Vermicelli, Spaghetti, Noodles, etc., Biscuits and Milk Flour; Other Cereals.

**Paper and Pulp Testing.** By E. SUTERMEISTER, S. B., Westbrook, Me. Paper; Physical Tests; Chemical Tests; Wood Pulp.

**Aliphatic Acids.** By HUGO SCHLATTER, M. S., Wilmington, Del. General Reactions; Acetic Acid; Vinegar; Homologues of Acetic Acid; Malic Acid; Tartaric Acid; Tartrates; Citric Acid; Citrates; Lactic Acids.

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**Special Characters and Modes of Examining Fats, Oils and Waxes.** By C. AINSWORTH MITCHELL, M. A., F. I. C., London. Olive Oil Group; Rape Oil Group; Cottonseed Oil Group; Linseed Oil Group; Castor Oil Group; Cacao Butter Group; Lard Oil Group; Tallow and Butter Group; Whale Oil Group; Sperm Oil Group; Beeswax Group.

**Butter Fat.** By CECIL REVIS and E. R. BOLTON, London. General; Qualitative Tests; Butter; Estimations; Preservatives; Margarine; Hardened Fats; Vitamines; Ghee.

**Lard.** By GEORGE A. REITZ, B. Sc., Ph. C., Philadelphia.

**Linseed Oil.** By GLENN H. PICKARD, Minneapolis. General; Iodine; Hexabromide Test; Other Tests and Methods, Oxygen Absorption; Polymerised Oil; Refining; Air Treated Oils; Boiled Oil; Effect of Storage; Detection of Adulterants.

**Higher Fatty Acids.** Revised by H. E. COX, M. Sc., Ph. D., F. I. C., Newport. Characteristics; General Properties; Separation of Mixed Fatty Acids; Palmitic Acid; Stearic Acid; Oleic Acid; Sebatic Acid; Elaidic Acid; Sulfopholeic Acid.

**Soaps.** By ELBERT C. LATHROP, A. B., Ph. D., Philadelphia. General; Detergent Action; Raw Materials; Alkalies and Fillers Varieties; A. C. S. Methods; Separation of Unsaponified Matters; Phenols; Examination for Special Constituents; Soap Powders, etc.; Interpretation of Analyses; Specifications.

**Glycerin.** By J. W. LAWRIE, Ph. D., Wilmington. Uses for Glycerin; Pharmacopoeia Test; Qualitative Test; Estimation; Specific Gravity; Refractive Index; Boiling Points; Analysis of Crude Glycerin; Analysis of Refined Glycerin; Pure Glycerin; Moisture in Glycerin; Foos; Polyglycerins; Fermentation Glycerin; Glycerin in Wines; Physical Constants.

**Wool-fat, Wool-grease, Suint, Degras.** By AUGUSTUS H. GILL, PH. D., SC. D., Boston. Wool-fat; Quantitative Methods; Lanolin; Distilled Wool-grease; Degras; Cloth Oils.

**Sterol Alcohols.** By JOHN ADDYMAN GARDNER, M. A., F. I. C., London. Cholesterol; Vegetable Sterols; Sources of Sterols; Phytosterol; Amorphous Sterols; Extraction of Sterols; Colorimetric Methods.

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**Bitumens.** By SAMUEL P. SADTLER, PH. D., L. L. D., Philadelphia. Natural Gas; Petroleum; Distillation of Petroleum; Naphtha; Kerosene; Gas Oil; Lubricating Oils; Lubricating Greases; Petrolatum; Paraffin; Asphalt; Asphalt Fluxes; Asphalt Pavings; Roofing Papers; Bibliography.

**Napthalene and Its Derivatives.** By W. A. DAVIS, B. SC., A. C. G. I., Rock Ferry. Napthalene; Napthalene Oils; Napthols; Napthol Ethers; Napthol Sulphonic Acids; Bibliography.

**Anthracene and Its Associates.** By JOHN H. SACHS, PH. D., Wilmington. Anthracene; Anthraquinone; Phenanthrene; Carbazol; Compounds with Picric Acid; Valuation of Anthracene; Bibliography.

**Phenols.** By J. BENNETT HILL, PH. D., Philadelphia. Monohydric Phenols; Phenol; Cresols; Xylenols; Commercial Carbolic Acids; Dip and Flotation Oils; Creosote; Cresylic Acid Disinfectants; Dihydric Phenols; Guaiacol; Wood Creosote; Trihydric Phenols; Bibliography.

**Aromatic Acids.** By EDWARD HORTON, B. SC., London. Sulphonated Phenols; Napthol Sulphonic Acids; Benzoic Acid; Metallic Benzoates; Benzoic Esters; Benzoic Aldehyde; Oil of Bitter Almonds; Saccharin; Cinnamic Acids; Cinnamic Esters; Cinnamic Aldehydes; Oil of Cinnamon; Coumarin; Gum Benzoin; Peruvian Balsam; Tolu Balsam; Liquid Storax; Salicylic Acid; Metallic and Alkaloidal Salicylates; Salicylic Esters; Derivatives of Salicylic Acid; Homologues of Salicylic Acid; Hydroxy-toluic Acids; Dihydroxy-benzoic Acids; Vanillin; Bibliography.

**Gallic Acid and Its Allies.** By W. P. DREAPER, O. B. E., F. I. C., London. Gallic Acid; Esters and Derivatives of Gallic Acid; Pyrogallol; Bibliography.

**Phthalic Acid and the Phthaleins.** By W. A. DAVIS, B. SC., A. C. G. I., Rock Ferry. Phthalic Acids; Phthalic Anhydrides; Phthaleins; Phenolphthalein; Indicators; Bibliography.

**Modern Explosives.** By A. MARSHALL, F. I. C., Kirkee, India. Introductory; Cellulose Nitrates; Examination of Nitrocellulose; Nitrostarch; Nitroglycerin; Separation of Nitro Aromatic Compounds; Picric Acid;

Picrates; Dinitrophenol; Nitrotoluenes; Trotyl or T. N. T.; Nitrochlorobenzenes; Nitronaphthalenes; Tetranitromethylaniline (Tetryl); Mercury Fulminate; Gelatinizers and Stabilizers; Diphenylamine; Moisture in Explosives; Analysis of Complex Explosives; Fireworks; Detonators; Abel Heat Test; U. S. Directions for Abel Test; Significance of Heat Tests; Fume Tests; Quantitative Tests; Chemical Methods; Bibliography.

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**Resins.** By ERNEST J. PARRY, B. Sc., F. I. C. Chemical Composition; Resin Esters; Resin Alcohols; Resinolic Acids; Resenes; Characters of Resins; Commercial Resins; Acaroid; Amber; Colophony; Common Resin; Colophonates; Dry Distillation; Rosin Spirit; Rosin Oil; Detection of Adulterants; Copals; Sandarac; Mastic; Dammar Resin; Dragon's Blood; Guaiacum; Shellac; Oleoresins; Balsams; Common Turpentine; French Turpentine; Venice Turpentine; Larch Turpentine; Canada Balsam, Balsam of Copaiba; Copaiba; Capivi; Oleoresin of Cubebs; Capsicin; Gum Resins; Ammoniacum; Asafoetida; Elemi; Myrrh; Bdellium; Gamboge; Frankincense; Sagapenum; Ambergris; Civet; Castor; Scammony Resin; Podophyllum Resin; Jalap Resin.

**India Rubber, Gutta-Percha, Balata and Allied Substances.** By JOHN B. TUTTLE, B. Sc. Polyterpenes; India-Rubber; Rubber Substitutes; Reclaimed Rubber; Gutta-Percha.

**Constituents of Essential Oils and Allied Substances.** By ERNEST J. PARRY, B. Sc., F. I. C. Hydrocarbons; Pentenes; Hemiterpenes; Cymene; Terpenes; Monocyclic Terpenes; Limolene; Terpinolene; Terpinene; Phellandrene; Sylvestrene; Dicyclic Terpenes; Pinene; Camphene; Borynlene; Fenchene; Thujene; Carene; Olefinic Terpenes; Constitution of the Dicyclic Terpenes; Sesquiterpenes; Cadinene; Caryophyllene; Santalene; Gurgunene; Zingiberene; Diterpenes and Polyterpenes; Estimation of Hydrocarbons; Alcohols; Alcohols of the Methane and Allied Series: Geraniol

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**General Character and Analysis of Essential Oils.** By ERNEST J. PARRY, B. Sc., F. I. C. Extraction of Essential Oils, Composition; General Characters; Analysis of Essential Oils; Detection of Sulphur Compounds in E. O.; Estimation of Free Acids in E. O., Estimation of Alcohols in E. O.; Estimation of Esters; Aldehydes; Ketones; Carbonyl Number; Iodine Value of Essential Oils; Methoxyl Values; Optical Activity; Refractive Indices; Adulteration of Essential Oils.

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**Writing, Stamping, Typing and Marking Inks.** By C. AINSWORTH MITCHELL, M. A., F. I. C., England. Inks; Examination of Writing Inks; Valuation of Writing Inks; United States Government Master Specification for Writing Ink; for Record and Copying Ink; for Red Ink; for Hectograph Ribbons; Modern Carbon Writing Inks; Chemical Examination of Ink in Writing; Type Writing Inks; Typewriter Ribbons; United States Government Master Specification for Typewriter Ribbons, for Ribbons for Computing and Recording Machines; Carbon Papers; Rubber-Stamp Inks; Marking Inks.

**Printing Inks.** By JOHN B. TUTTLE, B. Sc., New York. Types of Inks; Composition; Manufacture of Ink; Analysis; Separation of the Oil; Oil Constants; Hard Gums; Unsaponifiable Matter; Analysis of the Pigment; Blue Inks; Red Inks; Green Inks; Inks of Other Colours; Permanence to Light; Dyes and Lakes; Special Tests.

**Amines and Ammonium Bases.** By H. E. COX, M. Sc., Ph. D., F. C. I., England. Monamines; Hydrazines; Substituted Hydrazines.

**Analysis of Leather.** By A. E. CAUNCE, M. Sc., F. I. C., England. Physical Tests; Analysis of Vegetable-Tanned Leather; Analysis of Chrome Leather.

**Colouring Matters of Natural Origin.** By PROF. W. M. GARDNER, M. Sc., F. I. C., England. Indigo; Logwood; Natural Yellow Colouring Matters; Fustic; Weld; Turmeric; Gamboge; Saffron; Annatto; Red Dyestuffs; Orchil and Cudbear; Litmus; Madder; Redwoods; Alkanet; Safflower; The Colouring Matters in Flowers.

**Colouring Substances in Foods.** By WALTER E. MATHEWSON, Topeka, Kansas. Qualitative Examination of Food Products for Pigments; General Methods for the Qualitative Separation or Isolation of Soluble Colouring Matters; Identification of Colouring Substances; Quantitative Spectro-Colorimetry; Analysis of Commercial Food Colouring Materials.

**Benzene and Its Homologues.** By J. BENNETT HILL, Ph. D., Philadelphia. Properties of Benzene Hydrocarbons; Commercial Benzene; Thiophene; Toluene; Xylenes; Trimethylbenzenes. Para-Methyl-isopropyl Benzene. Commercial Benzols; Coal Tar Light Oils.

**Aniline and Its Allies.** By A. B. DAVIS, Cincinnati, Ohio. Aniline; Salts of Aniline; Aniline-Sulphonic Acids; Homologues of Aniline; Sulphonic Acids; Homologues of Aniline; Analysis of Commercial Xylidine (Flotation Grade); Aniline Oils; Estimation of Acetanilide and Phenacetin in Admixture; Commercial Method for the Analysis of Meta-Toluylene-Diamine; Method for the Analysis of Benzidine; for the Analysis of Toluidine; for the Analysis of Dianisidine.

**Naphthylamines, Pyridine, Quinoline and Acridine Bases.** By A. B. DAVIS, Cincinnati, Ohio. Naphthylamines and Their Allies; Alkyl- and Acyl-Naphthylamines; Pyridine Bases; Acridine and Its Allies.

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**Dyes and Colouring Matters.** By HANS EDWARD FIERZ-DAVID, D. SC., Zurich, Switzerland. Chemical Identification of the Different Groups; Historical Outline; Relations of Colouring Matters to Fibres; Classification of Dyes and Colouring Matters; Index of Names of Firms; Nitroso Colouring Matters and Nitro-colouring Matters; Azo Coloring Matters; Groups of Azo Dyes; Azo-colouring Matters. Mono-azo-dyes; Disazo Dyes; Primary Disazo Dyes; Secondary Disazo Dyes; Diamine Colours; Trisazo Dyes of Different Constitution; Tetrazo Dyes of Different Constitution; Stilbene Colouring Matters; Pyrazolone-dyes; Carbonium-dyes; Diphenylmethane Dyes; Triphenylmethane-dyes; Xanthenes; Acridine Dyes; Quinoline Dyes; Thiazoles (Primulines); Indamines, Indoanilines and Indophenols; Azines; Oxazine Colouring Matters; Thiazines; Sulphur Colouring Matters; Hydroxy-ketone Dyestuffs; Hydroxy-anthraquinones; Acid Anthraquinone Dyes; Anthraquinone Vat Colours; Indigoid Colouring Matters.

**The Synthetic Dyestuffs.** By A. W. JOYCE, PH. D., New York. Classification of the Synthetic Dyestuffs; The Nitroso Dyestuffs; The Nitro Dyestuffs; The Azo Dyestuffs; The Basic Azo Dyes; Acid Monazo Dyes; Monazo Dyes from Ortho-aminophenols; The Ice Colours; Pyrazoline Dyes; Acid Disazo Dyes; Substantive or Direct Cotton Dyes; Direct Cotton Dyes Derived from Diamines of the Benzene and Naphthalene Series; Direct Cotton Dyes Derived from J-acid and Its Derivatives; Dyes from Thiazole Bases; The Stilbene Dyes; Ketonimines; The Carbonium Colouring Matters; The Arylmethane Dyes; Diamino Derivatives; Triamino Derivatives; Amino-oxy-derivatives; Oxy-derivatives; Diphenyl-naphthyl Methane Dyes; Xanthene Dyes; The Amino Derivatives; The Hydroxy Derivatives; Amino-hydroxy Derivatives; Acridine Dyes; Quinoline Dyes; Thiazole Dyes; Indophenols and Indamines; Oxazine Dyes; Thiazine Dyes; Azine Dyes; Sulphur Dyes; Anthraquinone Dyes; Mordant-dyeing Anthraquinone Dyes; Anthraquinone Acid Dyes; Vat Dyes; Indigo and Indigoid Vat Dyes; Benzoquinone Vat Dyes; Identification of Azo Dyes; Reduction Products of Azo Dyes and the Dyes from Which They are Obtained; Physical and Chemical Properties of the Reduction Products from Azo Dyes.

**Analysis of Colouring Matters.** By HANS EDWARD FIERZ-DAVID, D. SC., Zurich, Switzerland and V. E. YARSLEY, D. SC., M. SC., A. I. C., General; Colour Standards and Colour Comparison; Aniline Lakes; Spectroscopic Investigation; Qualitative Investigation of Dyestuffs in Substance; Qualitative Investigation of Dyestuffs on Animal Fibre; Triphenylmethane Dyestuffs; General Procedure; Treatment of Mixtures; Qualitative Investigation of Dyestuffs on Vegetable Fibres; Preliminary Investigations; Chemical Reactions of the More Important Classes of Dyestuffs; Quantitative Analysis of Dyestuffs; Relative Methods; Absolute Methods; Titration with Hydrosulphite; Bibliography.

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**General Section on Alkaloids.** By T. M. SHARPE, M. SC. TECHN. A. I. C. London. Alkaloids of Alstonia Bark; Alkaloids of Areca or Betel Nut; Alkaloid of Barley Germs; Alkaloids of the Common Broom; Alkaloids of the Calabar Bean; Alkaloids of Delphinium; Alkaloids of Ephedra Spp.; Alkaloids of Ergot; Toxicological Investigation of Ergot; Alkaloids of Hemlock; Poisoning by Coniine and Hemlock; Assay of Hemlock and its Preparations; Alkaloids of Holarrhena Spp.; Alkaloids of Ipecacuanha; Alkaloids of Jaborandi; Alkaloids of Yellow Jasmine; Alkaloid of Laburnum and Furze; Alkaloids of Lobelia Inflata; Alkaloids of Lupuis; Alkaloids of Meadow Saffron (Colchicine); Assay of Colchicum; Toxicology of Colchicum; Alkaloids of Mescal Buttons; Alkaloid of Papaya; Alkaloids of Pegannum Harmala; Alkaloids of Yagé and Caapi; Alkaloids of Pepper; Analysis of Pepper; Alkaloids of Pomegranate; Alkaloids of the Potato, etc.; Alkaloids of Labadilla; Alkaloids of the Hellebores (Veratrum); Alkaloid of Yew; Alkaloids of Yolumba Bark; Alkaloids of Aspidosperma Quebracho Blanco; Bibliography.

**Aconite Alkaloids.** By FRANCIS H. CARR, C. B. E., F. I. C., London. Species of Aconite Plants; Constitution and Characters of the Aconite Bases; Aconitine; Salts of Aconitine; Chemical Reactions of Aconitine; Derivatives of Aconitine; Benzaconine; Aconine; Pyraconitine; Pyraconine; Amorphous Alkaloids of *A. Napellus*; Japaconitine; Indaconitine; Pseudo-aconitine; Bihhaconitine; Jesaconitine; Lycaconitine and Myoctonine; Lycoctonine; Myoctonine; Lapaconitine, Leptentrionaline and Cynoctine; Atisine; Assay of Aconite Root and its Preparations; Toxicology of Aconite; Toxicological Detection of Aconite; Pharmacology of Aconite.

**Berberine and Its Associates.** By E. HORTON, B. Sc. Berberine; Constitution of Berberine; Reactions and Detection of Berberine; Estimation of Berberine; Volumetric Methods; Gravimetric Method; Salts of Berberine; Oxyacanthine; Berbamine; Hydrastine; Estimation of Hydrastine; Hydrastis Rhizome; Hydrastinine; Salts of Hydrastinine; Canadine; Calumbin.

**Caffeine, Tea and Coffee.** By J. J. FOX, D. Sc., F. I. C. and P. J. SAGEMAN, F. I. C., London. Caffeine and its Allies; Caffeine, Theine or Trimethyl-xanthine; Salts of Caffeine; Assay of Caffeine Sodium Salicylate; Theobromine; Diuretin; Derivatives of Caffeine; Theophylline; Tea; Constituents of Tea; Analysis of Tea; Moisture in Tea; Ash; Isolation and Estimation of Caffeine; Tannin; Extract; Stalks; Essential Oil; Adulterations of Tea; Caper Tea; Maté Paraguay Tea; Coffee; Composition of Coffee; Caffetannic Acid; Cafféol; Coffee Berries; Analysis of Coffee and Coffee Mixtures; Coating and Glazing Substances; Ground Coffee; Commercial Chicory; Coffee Extracts; Kola; False Kola or Kola Bitter; Guarana; Bibliography.

**Cinchona Alkaloids.** By OLIVER CHICK, F. I. C., London. Cinchona Barks; Composition of Cinchona Barks; Assay of Cinchona Barks; Separation of Cinchona Bases; Titration of Cinchona Alkaloids; Cinchona Alkaloids; Constitution; General Properties of Cinchona Bases; Quinine; Detection and Estimation of Quinine; Salts of Quinine; Examination of Commercial Quinine Sulphate; Iron and Quinine Citrate; Tincture of Quinine; Quinine Tablets; Hydroquinine; Quinidine; Quinamine; Cinchonidine; Cinchonine; Amorphous Cinchona Bases; Alkaloids of *Remijia* Bark; Homoquinine; Bibliography.

**Cocaine.** By SAMUEL P. SADTLER, Philadelphia. Revised by NORMAN EVERS, B. Sc., F. I. C., London. Cocaine; Qualitative Tests; Toxicological Identification of Cocaine; Separation and Determination of Cocaine; Salts of Cocaine; Examination of Commercial Cocaine and its Salts; Decomposition Products of Cocaine; Bases allied to Cocaine; Cocaine Substitutes; Coca Leaves; Liquid Extract of Coca.

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FIFTH EDITION. REVISED AND IN PART REWRITTEN

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## PREFACE TO FIFTH EDITION.

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Since the publication of the Fourth Edition great progress has been made in all the analytical methods covered by this volume; hence, all articles have been practically rewritten to bring them up to date in the fullest sense of the word. Despite the effort to keep the volume of the same size as formerly by the elimination of all obsolete methods, the amount of new matter exceeds that which has been dispensed with. The general arrangement of the previous edition will, in the main, be continued throughout the whole series, as it has worked out well in practice.

Lactic acid is included with the so-called organic acids in this volume; the name of the group, however, has been changed to "Aliphatic Acids," which seems more appropriate.

Very little general discussion of the technology of substances treated is given. It is hoped, however, that there is enough description to make the analyst sufficiently conversant with the subject. To avoid duplicating methods where the same subjects are repeated in another closely allied section, fairly complete cross references are given to the sections wherein the referred method is more logically or appropriately given.

The U. S. Bureau of Standards tables for computing alcohol percentages have been introduced. These are more complete and satisfactory than those previously used. The constituents of fusel oil are treated more completely than previously, and much new matter has been introduced. The section on Sugars has been more enlarged than any other. This is, of course, due to the newer methods which have become important.

The section on Starch is adequately treated with reference to colloidal considerations. The section on Paper, while still concise, is brought up to date in a very satisfactory manner, the article being a good working guide for an analyst who does not devote all his time to the subject of paper.

As there did not seem to be enough time when this volume was first undertaken, to send abroad to Mr. Davis his Introduction for revision, Mr. Firman H. Thompson, Formerly Professor of Chemistry at the Delaware State University, and Chemical Director of the Delaware State Experiment Station, kindly consented to make such revision as seemed necessary, but did not wish his name affixed to the article, as the changes were relatively slight.

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# INTRODUCTION

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BY WILLIAM A. DAVIS, B. SC., A. C. G. I.

THE term Analysis, though originally meaning the separation or splitting up of a substance into its constituent parts, has now become greatly extended in its application, so that a process of chemical analysis may mean either:

A true analysis, or separation of a substance into its constituent parts;

A qualitative identification or recognition of a substance sought for; or

A quantitative estimation made with more or less accuracy of the composition of a substance.

When the quantitative analysis is limited to one or two important substances which constitute the valuable or active constituents of a more complex material, the analytical process is frequently called an *assay*. It is in this sense the term assay is employed throughout this work.

Very frequently the chemical examination of a substance includes the search for, or estimation of, impurities and foreign constituents accidentally present or purposely added. The nature of the foreign ingredients will, of course, largely depend upon that of the substance, and cannot be generally described. They may, however, be conveniently classified under the following heads:

Foreign substances naturally associated with the main substance, and not readily removed during the process of preparation. *Examples:* acetone in wood spirit; hydrogen cyanide in bitter-almond oil, and cresylic acid in carbolic acid.

Foreign substances introduced during the process of manufacture, and not subsequently (perfectly) eliminated. *Examples:* potassium cyanate and carbonate in commercial cyanide; sulphuric acid and lead salts in organic acids; alcohol in ether.

Foreign substances legitimately added in small quantity, to confer some special property on the main substance. *Examples:* mineral acids in hydrocyanic acid; alcohol in chloroform.

**Foreign** substances produced by the spontaneous change of the main substance. *Examples:* benzoic acid in bitter-almond oil; metaldehyde in aldehyde; ethyl acetate in tincture of iron acetate.

Adulterants purposely added to increase the weight or bulk, to confer some special property, or to conceal weakness or inferiority of the main substance. *Examples:* water in spirituous and vinous liquids; tartaric acid in citric acid; nitrobenzene in bitter-almond oil.

In the physical and chemical examination of organic materials many methods are employed, the details of which will be given under the proper heads, but the following general principles are frequently employed for the recognition and quantitative examination of such substances.

**A preliminary examination** of the leading characters of the substance, such as its colour, taste, odour, microscopic appearance and crystalline form.

A determination of the relative density of the substance, sometimes in the solid form, more frequently in the liquid condition, and occasionally in the state of vapour. The density of the solution of a substance is often a character of value.

Observations and operations connected with a *change in the physical state* of the substance, such as determinations of its melting and boiling points, and its behaviour on distillation.

A study of the *optical properties* of the substance, including its refractive and dispersive powers, absorption-spectrum, fluorescence, and action on a ray of polarised light.

A determination of the ultimate or *elementary composition* of the body.

The behaviour of the substance with ordinary *solvents*.

The behaviour of the substance with other *reagents*.

An examination of the substance for *inorganic* impurities.

The foregoing methods of examination are chiefly applicable to the recognition of comparatively pure compounds, but the principles involved are continually applied in the practical proximate analysis and chemical examination of organic materials. Thus, from the behaviour of the associated substances, when examined by one or more of the above methods, a practical recognition, determination, or separation of the constituents of the sample is effected.

It is not proposed to describe the whole of the above methods of examination in detail, as many of them are processes with the general

nature of which the user of this book is presumably acquainted. In most cases the outline of the method of examination is alone indicated, but exceptions are made in cases in which the same methods are not in general use in the analysis of inorganic substances. Sufficient working details for the use of any one versed in simple chemical manipulation are given under the special articles devoted to the examination of the various organic preparations employed in commerce.

### PRELIMINARY EXAMINATION

When the organic substance to be examined is of wholly unknown nature a judicious preliminary examination will often throw much light on its composition. The following points should not be lost sight of:

**Colour.**—The colours of organic bodies are not, as a rule, very characteristic, but there are some very remarkable exceptions. As a rule, blue vegetable colouring matters are rendered red by acids, and the blue colour is restored or changed to green by ammonia. Indigo is not affected. Vegetable yellows are generally turned brown by alkalies, and the colours restored by acids. The examination of the absorption-spectra of coloured organic substances often furnishes most valuable information (see page 34).

**Taste.**—This character must be observed with extreme caution, as many organic compounds are intensely poisonous. The safest way is to make a weak aqueous or alcoholic solution of the substance and taste a drop of the liquid cautiously. Acids are, as a rule, sour or astringent in taste. Alkaloids are usually bitter. The sugars and glycerin are sweet.

**Odour.**—The odour of organic compounds is often highly characteristic, and notably so in the case of the neutral alcoholic derivatives.

**Microscopic Appearance.**—In the case of solid bodies an examination under the microscope is often extremely useful. As a rule, the use of a high power is neither necessary nor desirable. The micro-polariscope affords a valuable means of identifying starches.

**Crystalline Form.**—This character is often of great service for the recognition of organic substances and especially as a test for purity. In the great majority of cases the crystals are too small or indistinct to admit of any goniometric determination, but the appearance of a substance under the microscope and especially its be-

haviour towards polarised light afford valuable evidence. Instances of the value of crystalline form as means of identification are to be found in the cases of cholesterol, salicylic acid, tartaric acid, and some of the alkaloids and their salts.

**Effect of Heat.**—The behaviour of organic substances on heating is often highly characteristic. Solids should be heated in a small, dry test-tube. It is well to make an experiment first on a piece of platinum foil, as a few substances explode violently when heated. On ignition in the air all organic substances other than those containing metals are completely consumed. Sometimes volatilisation occurs without darkening; in other cases, a more or less voluminous residue of carbon is left, which is sometimes burned away only with great difficulty. Nitrogenous organic compounds burn with a distinctive, unpleasant odour or when heated with alkalies or soda lime produce free ammonia, easily detected by the odour or by moist litmus paper. Salts of organic acids containing metals of the alkalies or alkaline earths usually leave these metals as carbonates on being ignited in the air. Hence the presence of carbonate in the ash indicates the previous presence of an organic acid. Volatile heavy metals, such as arsenic or mercury, are wholly driven off on igniting substances containing them, but most heavy metals remain on ignition either as oxides or in the metallic state.

The specific gravity, boiling and melting points, and other physical properties of the substance may be roughly noted as part of the preliminary examination, but these characters are referred to at greater length in the following sections.

### SPECIFIC GRAVITY OR RELATIVE DENSITY

The specific gravity of an organic solid or liquid is often a most valuable criterion of its identity or purity. Unlike the determination of the density of a vapour, it is frequently applicable to the accurate estimation of a substance in solution or in admixture with another body, and in other cases it may be used to discriminate between substances of the same percentage composition. It is, however, unadvisable to depend upon the specific gravity for quantitative estimations in solutions containing more than one constituent or in cases where the purity of the solution is doubtful as in the case of alcohol in alcoholic extracts. In such cases a purification of

the solution is necessary, usually by distillation, before determining the specific gravity.

The relative density of a solid or liquid is generally referred to water taken either as *unity* or as 1000. In stating specific gravities the temperature at which they are taken should also be given, it being assumed to be referred to water at the same temperature. It is better, however, to state the temperatures at which the masses of both the liquid and the water were determined as for instance  $60^{\circ}/60^{\circ}$  F. means they were both taken at  $60^{\circ}$  F. while  $60^{\circ}\text{F.}/4^{\circ}\text{C.}$  means the substance in question was taken at  $60^{\circ}$  F. while the water was taken at  $4^{\circ}$ . Both plans have their advantages, and, as no confusion can arise from such a course, the sp. gr. given in this work will be stated in either manner, according to convenience of expression or comparison.

**The specific gravity bottle** is the most generally serviceable means of taking the sp. gr. of solids and liquids. It should not be trusted to contain the amount of water marked on it, but should be filled with distilled water at the temperature at which the sample of liquid is to be compared, and the weight of contained water ascertained. The sp. gr. of the sample is found by dividing the weight of it which the bottle contains by the weight of water contained at the same temperature. When the liquid is miscible with water, the wet bottle may be rinsed out once or twice with a few drops of the sample; when the liquid is immiscible or nearly so with water, the bottle should be rinsed once or twice with alcohol and then with ether, the last traces of the latter being got rid of by a current of dry air from a bellows, or by sucking the ether-vapour from the warmed bottle by means of a glass tube. For the determination of liquids a pycnometer of the type shown in Fig. 1 is commonly used which permits of an accurate reading of the actual temperature of the liquid. In using this pycnometer the bottle should be filled well up the neck of the larger opening and cooled slightly below the temperature desired. The ground glass stopper carrying the thermometer is carefully inserted thus completely filling the vessel with the liquid and expelling the excess through the side tube. The temperature is now allowed to rise to the desired point and the excess liquid removed



FIG. 1.  
Pycnometer.

from the top of the capillary side tube with a piece of blotting paper. The ground glass cap is put on and after carefully drying the outside of the bottle the weight is taken.

The selection of the temperature of  $15.5^{\circ}$  ( $60^{\circ}$  F.) sometimes involves considerable practical inconvenience especially in the summer months. Squibb has introduced a urinometer for  $25^{\circ}$  ( $77^{\circ}$  F.) which, in the ordinary use of this instrument, is a much more convenient temperature. The current United States Pharmacopœia has adopted this temperature. Squibb has devised a bottle which eliminates the inconvenience of operating at a special temperature. The annexed description is from *Ephemeris*, January, 1897.



FIG. 2.

The bottle (Fig. 1) should hold 100 grm. of recently-boiled distilled water at  $20^{\circ}$  at about 58 on a scale of 0 to 100. In weighing the water into the bottle, the fine adjustment to 0.001 grm. is made by use of narrow strips of blotting-paper that will pass easily down the bore of the graduated stem. When the 100 grm. are in the bottle, and the column stands between 50 and 65 divisions of the scale, the stopper is put in, a leaden ring is placed on the neck, and the whole immersed in a bath of broken ice and water until the column of water comes to rest. It should then read at zero of the scale, or not much above it, and the reading should be noted. If it reads below zero, the bottle is too large, and the stopper part of the stem must be ground farther into the bottle neck, until the reading, on new trial, brings the column a little above zero. The bottle is then put into a bath at  $25^{\circ}$  and kept there, the bath being stirred,—until the column comes to rest, when it should read about 90 to 100 of the scale. Should it read above 100, while the lower limit is as far above the zero, the bottle is too small, and the end of the stopper must be ground off until the reading of the column is within the graduations at both ends of the scale.

With this bottle the sp. gr. can be taken at any of the temperatures of the standard unit volume to the sixth decimal place. For the construction of a simple thermostat enabling the temperature to be kept within a few thousandths of a degree for long periods, see Lowry, *Trans.*, 1905, 87, 1030, and *Trans. Faraday Soc.*, 1907, part iii. A description is given on page 57.

**Sprengel's Tube.**—A useful method of taking the sp. gr. of liquids, especially when but small quantities are at disposal, is that of Sprengel (*Jour. Chem. Soc.*, 1875, 26, 577), in which a small U-shaped apparatus terminating in horizontal capillary tubes is substituted for the ordinary bottle. It may be easily filled and the regulation of the quantity of contained liquid is also easily effected. The

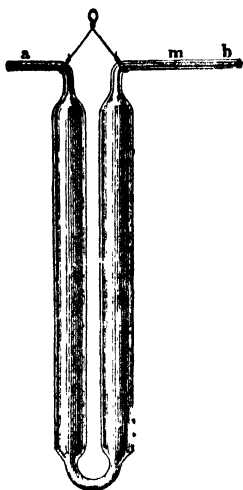


FIG. 3.

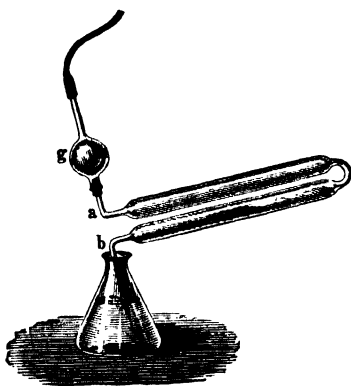


FIG. 4.

results are of a high degree of accuracy. Sprengel's tube has the advantage that it can be used for ascertaining the sp. gr. at the b. p. of water and is particularly valuable for determining the sp. gr. of fats which are solid at lower temperatures but become liquid below the boiling point of water. It consists (Fig. 3) essentially of a thin glass U-tube terminating in two capillary ends bent at right angles and each provided with a ground cap. One of these capillary tubes must have a smaller calibre than the other—not larger than 0.25 mm. The larger tube should bear a mark at *m*. The tube is filled by immersing *b* in the liquid under examination, connecting the smaller end with a large glass bulb, and applying suction to the latter by means of a rubber tube, as shown in Fig. 3. If now the rubber tube be closed, the glass tube will fill automatically. It is placed in water, the ends being allowed to project, and the water is brought to the proper temperature. The Lowry thermostat may be used here with advantage. A conical flask may also be used to

contain the water, the ends of the Sprengel tube being supported by the neck. The mouth of the flask should be loosely covered. As the liquid expands in the Sprengel tube it will drop from the larger orifice. When this ceases, the liquid is adjusted to the mark at *m*. If beyond the point, a little may be extracted by means of a roll of filter-paper. The tube is then taken out of the bath, the caps adjusted, the whole thoroughly dried, allowed to cool, and weighed. The same operation having been performed with distilled water, the calculation of the sp. gr. is made as usual.

For more elaborate directions as to use of this apparatus see Ostwald's *Physico-chemical Measurements* or Findlay's *Practical Physical Chemistry*. For the rapid determination of the sp. gr. of liquids, especially saturated solutions, the Meyerhoffer-Saunders pipette, modified by Bousfield, is convenient (see Lowry, *Trans.*, 1906, 89, 1036).

**Hydrometers** are instruments the use of which is too well known to require detailed description. Care should be taken in making accurate observations to read either from the top, bottom, or centre of the meniscus, according to the manner in which the instrument is graduated. Attention should also be paid to the temperature of the liquid during the observation.

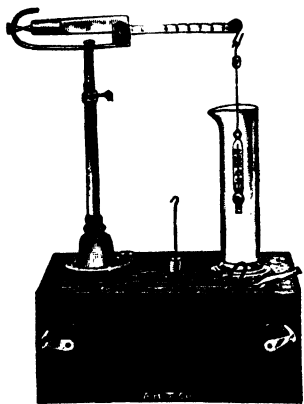


FIG. 5.—Westphal balance.

The graduation of hydrometers, even when sold by well-known firms, is often far from accurate; hence the indications of such instruments should be carefully verified.

The accuracy of hydrometer-densities has been questioned in the case of milk and other liquids containing suspended particles, but the experiments of L. Siebold (*Analyst*, 1879, 4, 180) show that the indications of the hydrometer in such cases agree with those obtained by the sp. gr. bottle. Hydrometers are graduated to read in direct or according to an arbitrary scale devised for some special purpose.

**Twaddell's hydrometer** is applicable only to liquids heavier than water. It is so graduated that 200 divisions on the scale represent

the densities between 1 and 2, each division thus representing a difference in sp. gr. of 0.005. Twaddell readings are converted into sp. gr. by multiplying by 0.005 and adding 1.

The *Westphal balance*, Fig. 5, while not so accurate as the pycnometer or Sprengel tube is sufficiently so for most purposes and has the advantage of being more rapid. It consists of a scale beam, from one end of which a thermometer sinker is suspended. This is so adjusted that the beam balances when the sinker is suspended in water. When suspended in the liquid whose density is to be determined the loss of weight of the sinker is compensated by weights on the graduated beam. The sp. gr. is then read directly from the beam.

**Baumé's hydrometer** is not commonly used in England, except for ascertaining the sp. gr. of saccharine solutions. But in the United States it is probably used more than any other hydrometer in commercial work. There are two Baumé scales, one for liquids lighter than water and one for liquids heavier than water. The scale for liquids lighter than water was constructed by marking the point to which the spindle sank in a 10 per cent. solution of sodium chloride as 0 and in pure water as 10 and extending this scale. For liquids heavier than water the point to which the spindle sank in a 15 per cent. salt solution was marked 15 and in pure water as 0 and extending the scale. It is obvious that while the divisions on the Baumé scale are the same size the value of a Baumé degree in absolute density varies and involves some calculation for its conversion.

Much confusion and irregularity exist as to the scales of Baumé hydrometers commonly sold. C. F. Chandler (*Proc. National Acad. Sci.*, 1881, 3) found 36 different scales in use, many of them incorrect. This conversion is usually accomplished by reference to tables. The formulæ for converting Baumé readings into sp. gr. according to the United States Bureau of standards are as follows:

$$\begin{aligned} \text{For liquids lighter than water: degrees Baumé,} \\ = \frac{140}{\text{sp. gr. at } 60^{\circ} \text{ F.}} - 130 \end{aligned}$$

$$\begin{aligned} \text{For liquids heavier than water: degrees Baumé,} \\ = 145 - \frac{145}{\text{sp. gr. at } 60^{\circ} \text{ F.}} \end{aligned}$$

$$\text{For liquids heavier than water: sp. gr.} = \frac{145}{145 - n} \text{ at } 60^{\circ} \text{ F.}$$

For liquids lighter than water: sp. gr. =  $\frac{145}{130 + n}$  at 60° F.

The so-called "rational" hydrometer, proposed originally by Kolb in France, but most widely used in Germany, is based on the following principle:

If a hydrometer sinks in water to the mark 0°, and in a liquid *D* having a sp. gr. *d* to *n*°, then, as in each case the weight of the hydrometer *W* is equal to the weight of the liquid displaced, we have—

Wt. of the volume of water displaced by the hydrometer = *W*

Wt. of the same volume of liquid *D* = *dW*

Wt. of water displaced by *n* divisions of the scale = *n*

Wt. of same volume of liquid *D* = *dn*

For the weights *dW* and *W* to differ by *nd*,

$$dW - W = nd.$$

$$\therefore d = \frac{W}{W - n} \dots \dots (1)$$

Kolb calibrated his hydrometer by reference to "pure sulphuric acid of sp. gr. 1.842 at 15°." The point to which the hydrometer sank in the acid was indicated as 66° Bé., with this method of calibration, from the above formula (1),

$$d = \frac{144.3}{144.3 - n}$$

Although Kolb's system of calibration was based on an incorrect value for the sp. gr. of pure sulphuric acid (see Lunge, *op. cit.*), (the sp. gr. of 100% H<sub>2</sub>SO<sub>4</sub> at 15°/4° being 1.8357), this method of calibration has been generally adopted in Germany, and was used for a time in the United States.

When the Baumé hydrometer is calibrated by reference to a 10% solution of pure sodium chloride (1 grm. in 9 grm. water) the following formula is obtained at 15°.

$$d = \frac{146.3}{146.3 - n}$$

This method of calibration is known as that of Gerlach.

The Manufacturing Chemists Association of the United States of America in 1898 adopted (*J. Soc. Chem. Ind.*, 1898, 17, 45) another method of calibration; in this scale "66° Bé." refers to sulphuric acid of sp. gr. 1.835 at 15°/4° not because this is the highest obtainable strength, but because this is the sp. gr. of the acid, handled as "66° oil of vitriol" in commerce, which contains 93.5% of H<sub>2</sub>SO<sub>4</sub> by weight.

TABLE I

Comparison of Different Baumé Hydrometers with True Sp. Gr.

*For Heavy Liquids*

Degrees	Rational scale	Gerlach scale	American scale		Degrees	Rational scale	Gerlach scale	American scale	
	$d = \frac{144.3}{144.3 - n^\circ}$	$d = \frac{146.3}{146.3 - n^\circ}$	$d = \frac{145}{145 - n^\circ}$	M. C. A. at $15^\circ/4^\circ$		$d = \frac{144.3}{144.3 - n^\circ}$	$d = \frac{146.3}{146.3 - n^\circ}$	$d = \frac{145}{145 - n^\circ}$	M. C. A. at $15^\circ/4^\circ$
	at $15^\circ$	at $15^\circ$	at $60^\circ$ F.			at $15^\circ$	at $15^\circ$	at $60^\circ$ F.	
1	1.007	1.0068	1.007	1.005	34	1.308	1.3015	1.306	1.309
2	1.014	1.0138	1.014	1.011	35	1.320	1.3131	1.318	1.317
3	1.022	1.0208	1.021	1.023	36	1.332	1.3250	1.330	1.334
4	1.029	1.0280	1.028	1.029	37	1.345	1.3370	1.343	1.342
5	1.037	1.0353	1.036	1.036	38	1.357	1.3494	1.355	1.359
6	1.045	1.0426	1.043	1.043	39	1.370	1.3619	1.368	1.368
7	1.052	1.0501	1.051	1.050	40	1.383	1.3746	1.381	1.386
8	1.060	1.0576	1.058	1.057	41	1.397	1.3876	1.394	1.395
9	1.067	1.0653	1.066	1.064	42	1.410	1.4009	1.408	1.413
10	1.075	1.0731	1.074	1.071	43	1.424	1.4134	1.422	1.422
11	1.083	1.0810	1.082	1.086	44	1.438	1.4281	1.436	1.441
12	1.091	1.0890	1.090	1.093	45	1.453	1.4421	1.450	1.451
13	1.100	1.0972	1.098	1.100	46	1.468	1.4564	1.465	1.470
14	1.108	1.1054	1.107	1.107	47	1.483	1.4710	1.480	1.480
15	1.116	1.1138	1.115	1.114	48	1.498	1.4860	1.495	1.500
16	1.125	1.1224	1.124	1.122	49	1.514	1.5012	1.510	1.510
17	1.134	1.1310	1.133	1.136	50	1.530	1.5167	1.526	1.531
18	1.142	1.1398	1.142	1.143	51	1.546	1.5325	1.543	1.541
19	1.152	1.1487	1.151	1.150	52	1.563	1.5487	1.559	1.561
20	1.162	1.1578	1.160	1.158	53	1.580	1.5652	1.576	1.573
21	1.171	1.1670	1.169	1.172	54	1.597	1.5820	1.593	1.594
22	1.180	1.1763	1.179	1.179	55	1.615	1.5993	1.611	1.616
23	1.190	1.1858	1.188	1.186	56	1.634	1.6169	1.629	1.627
24	1.200	1.1955	1.198	1.201	57	1.652	1.6349	1.648	1.650
25	1.210	1.2053	1.208	1.208	58	1.671	1.6533	1.667	1.661
26	1.220	1.2153	1.218	1.216	59	1.691	1.6721	1.686	1.683
27	1.231	1.2254	1.229	1.231	60	1.711	1.6914	1.706	1.705
28	1.241	1.2357	1.239	1.238	61	1.732	1.7111	1.726	1.727
29	1.252	1.2462	1.250	1.254	62	1.753	1.7313	1.747	1.747
30	1.263	1.2560	1.261	1.262	63	1.774	1.7520	1.768	1.767
31	1.274	1.2677	1.272	1.269	64	1.796	1.7731	1.790	1.793
32	1.285	1.2788	1.283	1.285	65	1.810	1.7948	1.812	1.814
33	1.297	1.2901	1.295	1.293	66	1.842	1.8171	1.835	1.835

*American Scales.*

1. sp. gr. =  $\frac{145}{145 - n^\circ}$  at  $60^\circ$  F. See tables calculated by Emery,

*J. Amer. Soc.*, 1899, 21, 117.

2. Manufacturing Chemists Association of the U. S. A., *J. Soc. Chem. Ind.*, 1898, 17, 45.<sup>1</sup>

Degrees 1-2	$\Delta$ in sp. gr. = 0.006	Degrees 10-11	$\Delta$ = 0.015
2-3	$\Delta$ in sp. gr. = 0.012	11-12	$\Delta$ = 0.007
3-4	$\Delta$ in sp. gr. = 0.006	12-13	$\Delta$ = 0.007
4-5	$\Delta$ in sp. gr. = 0.007		
	Degrees 19-20	$\Delta$ = 0.008	
	20-21	$\Delta$ = 0.014	
	21-22	$\Delta$ = 0.007	

and so on.

In the upper part of the scale the differences are still more peculiar; *e. g.*:

Degrees 45-46	$\Delta$ = 0.019
46-47	$\Delta$ = 0.010
47-48	$\Delta$ = 0.020
Degrees 54-55	$\Delta$ = 0.022
55-56	$\Delta$ = 0.011
56-57	$\Delta$ = 0.011
58-59	$\Delta$ = 0.022

It would appear from the statement in the *J. Soc. Chem. Ind.*, 1905, 24, 782 that the M. C. A. has now adopted the scale of the (American) General Chemical Company (see page 10).

The table on page 11 gives a comparison of the different scales with true sp. gr. (at 15°) for liquids heavier than water. As Göckel has pointed out (*Zeit. angew. Chem.*, 1903, 562), Baumé hydrometers should have inscribed on them not only the temperature for which they are calibrated, but also the temperature of the water used for comparison; it should also be stated whether the weights are referred to normal pressure (760 mm.) or to vacuum. In Germany, the Imperial Commission of Normal Standards (Kais. Normal Eichungskommission, 1904, Heft 5) has recently thoroughly investigated the relation of degrees Baumé ("rational" scale) to true specific gravity. They give the following table (Table II.) for the transformation of sp. gr. at 15°/4° into degrees. Bé. (rational).

<sup>1</sup> The scale adopted by the (American) Manufacturing Chemists Association is a most irrational one as the values of sp. gr. plotted against degrees Baumé do not give a properly continuous curve. The non-scientific character of this scale is at once visible on considering the differences of sp. gr. for successive degrees Baumé; *e. g.*:

TABLE II

Transformation of Sp. Gr. at 15°/4° into Degrees Baumé of the Rational Scale

S 15/4	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0.99										-0.018
1.00	0.126	0.270	0.414	0.557	0.700	0.843	0.986	1.128	1.270	1.412
01	1.553	1.694	1.835	1.976	2.117	2.257	2.397	2.536	2.675	2.814
02	2.953	3.091	3.229	3.367	3.505	3.643	3.780	3.917	4.053	4.189
03	4.325	4.461	4.596	4.731	4.866	5.001	5.135	5.269	5.403	5.537
04	5.671	5.804	5.937	6.070	6.202	6.334	6.466	6.598	6.729	6.860
1.05	6.991	7.122	7.252	7.382	7.512	7.642	7.771	7.900	8.029	8.158
06	8.287	8.415	8.543	8.671	8.798	8.925	9.052	9.179	9.306	9.432
07	9.558	9.684	9.809	9.934	10.059	10.184	10.309	10.433	10.557	10.681
08	10.805	10.929	11.052	11.175	11.298	11.421	11.543	11.665	11.787	11.909
09	12.030	12.151	12.272	12.393	12.514	12.634	12.754	12.874	12.994	13.114
1.10	13.233	13.352	13.471	13.590	13.708	13.826	13.944	14.062	14.179	14.296
11	14.413	14.530	14.647	14.764	14.880	14.996	15.112	15.228	15.343	15.458
12	15.573	15.688	15.803	15.917	16.031	16.145	16.259	16.373	16.486	16.599
13	16.712	16.825	16.938	17.050	17.162	17.274	17.386	17.498	17.610	17.721
14	17.832	17.943	18.054	18.164	18.274	18.384	18.494	18.604	18.713	18.822
1.15	18.931	19.040	19.149	19.258	19.366	19.474	19.582	19.690	19.798	19.905
15	20.012	20.119	20.226	20.333	20.439	20.545	20.651	20.757	20.863	20.969
17	21.074	21.179	21.284	21.389	21.494	21.599	21.703	21.807	21.911	22.015
18	22.110	22.222	22.325	22.428	22.531	22.634	22.737	22.839	22.941	23.043
19	23.145	23.247	23.349	23.450	23.551	23.652	23.753	23.854	23.955	24.055
1.20	24.155	24.255	24.355	24.455	24.554	24.653	24.752	24.851	24.950	25.049
21	25.148	25.246	25.344	25.442	25.540	25.638	25.736	25.834	25.931	26.028
22	26.125	26.222	26.319	26.415	26.511	26.607	26.703	26.799	26.895	26.990
23	27.085	27.180	27.275	27.370	27.465	27.560	27.655	27.749	27.843	27.937
24	28.031	28.125	28.219	28.312	28.405	28.498	28.591	28.684	28.777	28.869
1.25	28.961	29.053	29.145	29.237	29.329	29.420	29.512	29.603	29.694	29.785
26	29.876	29.967	30.058	30.149	30.239	30.329	30.419	30.509	30.599	30.688
27	30.777	30.866	30.955	31.044	31.133	31.222	31.311	31.400	31.488	31.576
28	31.664	31.752	31.840	31.928	32.015	32.102	32.189	32.276	32.363	32.450
29	32.537	32.624	32.711	32.797	32.883	32.969	33.055	33.141	33.227	33.312
1.30	33.397	33.482	33.567	33.652	33.737	33.822	33.907	33.991	34.075	34.159
31	34.243	34.327	34.411	34.495	34.579	34.662	34.745	34.828	34.911	34.994
32	35.077	35.160	35.243	35.325	35.407	35.489	35.571	35.653	35.735	35.817
33	35.890	35.981	36.062	36.143	36.224	36.305	36.386	36.467	36.548	36.628
34	36.708	36.788	36.868	36.948	37.028	37.107	37.187	37.267	37.346	37.425
1.35	37.504	37.583	37.662	37.741	37.820	37.898	37.977	38.056	38.134	38.212
36	38.290	38.368	38.446	38.524	38.601	38.678	38.755	38.832	38.909	38.986
37	39.063	39.140	39.217	39.294	39.370	39.446	39.522	39.598	39.674	39.750
38	39.816	39.902	39.978	40.053	40.128	40.203	40.278	40.353	40.428	40.503
39	40.578	40.652	40.727	40.801	40.875	40.949	41.023	41.097	41.171	41.245
1.40	41.318	41.392	41.466	41.539	41.612	41.685	41.758	41.831	41.904	41.977
41	42.040	42.122	42.194	42.266	42.338	42.410	42.482	42.554	42.626	42.698



Great care must be exercised in expressing or interpreting results in the Baumé scale as, owing to the many different systems in use, confusion may easily arise.

The values of sp. gr. corresponding with degrees Baumé in the tables given by the United States Department of Agriculture, Bulletin No. 65 (1902), Table VI, and Bulletin No. 107 (1907), are apparently degrees of the rational scale,

$$d = \frac{144.3}{144.3 - n}$$

TABLE III  
Comparison of Degrees of Baumé Hydrometer for Light Liquids with Sp. Gr.

Degrees Baumé	Sp. gr. = 140	Sp. gr. = 146	Degrees Baumé	Sp. gr. = 140	Sp. gr. = 146	Degrees Baumé	Sp. gr. = 140	Sp. gr. = 146
	$\frac{130 + n}{\text{at } 60^{\circ} \text{ F.}}$	$\frac{136 + n}{\text{at } 12.5^{\circ}}$		$\frac{130 + n}{\text{at } 60^{\circ} \text{ F.}}$	$\frac{136 + n}{\text{at } 12.5^{\circ}}$		$\frac{130 + n}{\text{at } 60^{\circ} \text{ F.}}$	$\frac{136 + n}{\text{at } 12.5^{\circ}}$
10	1.0000	1.0000	27	0.8917	0.8957	44	0.8047	0.8111
11	0.9929	0.9932	28	0.8861	0.8902	45	0.8000	0.8066
12	0.9859	0.9865	29	0.8805	0.8848	46	0.7955	0.8022
13	0.9790	0.9799	30	0.8750	0.8795	47	0.7910	0.7978
14	0.9722	0.9733	31	0.8696	0.8742	48	0.7865	0.7935
15	0.9655	0.9669	32	0.8642	0.8690	49	0.7821	0.7892
16	0.9589	0.9605	33	0.8589	0.8639	50	0.7778	0.7849
17	0.9524	0.9542	34	0.8537	0.8588	51	0.7735	0.7807
18	0.9459	0.9480	35	0.8485	0.8538	52	0.7692	0.7766
19	0.9396	0.9420	36	0.8434	0.8488	53	0.7650	0.7725
20	0.9333	0.9359	37	0.8383	0.8439	54	0.7609	0.7684
21	0.9272	0.9299	38	0.8333	0.8391	55	0.7568	0.7643
22	0.9211	0.9241	39	0.8284	0.8343	56	0.7527	0.7604
23	0.9150	0.9183	40	0.8235	0.8295	57	0.7487	0.7565
24	0.9091	0.9125	41	0.8187	0.8249	58	0.7447	0.7526
25	0.9032	0.9068	42	0.8140	0.8202	59	0.7407	0.7487
26	0.8974	0.9012	43	0.8092	0.8156	60	0.7368	0.7449

Baumé hydrometers for liquids lighter than water are graduated in two ways:

1. The point to which the spindle sinks in a solution of 1 gram. of common salt in 9 gram. of water at  $12.5^{\circ}$  is called  $0^{\circ}$  and the point corresponding with pure water is called  $10^{\circ}$ . The degrees so obtained are repeated throughout the scale. This graduation gives:

$$\text{Sp. gr.} = \frac{145.88}{135.88 + n^{\circ}}; \text{ or approximately}$$

$$\text{Sp. gr.} = \frac{146}{136 + n^{\circ}}$$

2. In America (see tables given by Emery, *loc. cit.*) the divisions of the scale are obtained from the formula:

$$\text{Sp. gr.} = \frac{140}{130 + n^{\circ}} \text{ at } 60^{\circ} \text{ F.}$$

The table given on page 15 summarises the results of both methods of graduation.

**Cartier's Hydrometer.**—On this,  $22^{\circ}$  corresponds with  $22^{\circ}$  Bé., but above and below this point the degrees are diminished in the ratio of 16 to 15.

**Beck's Hydrometer.**—The zero point corresponds to the sp. gr. of water and  $30^{\circ}$  to sp. gr. 850, the scale being divided into equal parts above and below the zero point, as far as desirable.

Other hydrometers are described in the section on *sugars*.

Unfortunately, much confusion has crept into the mode of stating sp. gr. Thus, if a liquid be stated to have a sp. gr. 0.7185 at  $17.5^{\circ}$ , there is no certainty as to what is intended. It may be meant that a bottle which holds 100 grm. of water at  $17.5^{\circ}$  holds only 71.85 grm. of the liquid, or the bottle may hold 100 grm. of water at  $15.5^{\circ}$  ( $60^{\circ}$  F.), at  $15.0^{\circ}$ , at  $4^{\circ}$ , or at  $0^{\circ}$ . In many instances it is uncertain whether the recorded sp. gr. refers to a comparison with an equal volume of water at the same temperature as that at which the liquid was weighed or at any one of the temperatures just given. As a rule, when the sp. gr. of a substance is stated to have a given value at  $15.5^{\circ}$  ( $60^{\circ}$  F.), it may be regarded as probable that the unit of water was weighed at the same temperature, but in other cases it is not certain what is meant.

The sp. gr. of *organic solids* is best taken by introducing some fragments or powder into a sp. gr. bottle and ascertaining the weight. The bottle is next filled with water, petroleum, or some liquid of known density having no solvent action on the solid to be examined, and the weight is then again observed. The increase gives the weight of contained liquid, which divided by its known sp. gr., gives its volume. This subtracted from the known capacity of the bottle gives the volume of the solid, which, divided into its weight, gives the sp. gr. compared with water as unity. Care must be taken to avoid the adherence of air bubbles to the solid. Agitation will generally suffice to remove them.

In many cases the Blount or Schumann bottle used in the examina-

tion of cement may be with advantage employed with a suitable solvent.

Hager has described (*Analyst*, 1876, 4, 206), a method of ascertaining the sp. gr. of fats and similar bodies, by diluting alcohol or strong ammonia with water until suspended fragments of the substance remain in equilibrium in any part of the liquid at the standard temperature. The sp. gr. of the liquid is then taken, being the same as that of the solid. This is an adaptation of the well known method used in ascertaining the sp. gr. of minerals.

**Vapour-densities.**—The determination of the vapour-density of an organic substance often furnishes confirmation of its formula. In all cases in which decomposition of the substance does not occur, the density of the vapour, compared with that of hydrogen at the same temperature and pressure, is one-half the molecular weight.

The vapour-density of a volatile liquid is most rapidly ascertained by means of the method devised by Victor Meyer. The molecular weight of a non-volatile substance can be ascertained by measuring the rise of b. p. or depression of the freezing point of a suitable solvent in which a known amount of the substance is dissolved. For details of these methods see any treatise on practical physical chemistry; for example, Ostwald and Luther's *Physico-chemical Measurements* or Findlay's *Practical Physical Chemistry*.

## OBSERVATIONS OF CHANGES OF PHYSICAL STATE

The m. p. of an organic substance is best ascertained by heating a little of the substance in a capillary tube sealed at one end and about three inches long and 0.01 to 0.02 in diameter; such tubes are readily made by drawing out a test-tube in a blow-pipe flame. The tube containing the substance is placed at the side of the bulb of a thermometer, so as to adhere to it, and heated in a small beaker of strong sulphuric acid, the temperature of which is gradually raised by means of a small burner placed beneath. In order to make the reading of the m. p. as sharp as possible the temperature is raised only very slowly just before the substance melts. After melting, the substance should be allowed to solidify and the m. p. again taken. It must be borne in mind that although a pure substance generally melts quite sharply at a definite temperature (within 0.5°) the m. p. is much lowered and rendered indefinite by the presence of a small

quantity of impurity. *The m. p. is only of value, therefore, in characterising a substance which has been carefully purified.*<sup>1</sup>

The **subliming point** of an organic body is sometimes an important characteristic, but its value depends much on the manner of making the observation. A. Wynter Blyth recommends the following method: A porcelain crucible about 3 in. in diameter is nearly filled with mercury (or, for high temperatures, fusible metal). A minute quantity of the substance to be examined is placed on a thin disc of microscopic covering glass, which is floated on the mercury, and covered with a glass ring (cut from tubing), on which is placed a second disc so as to form a closed shallow cell. The porcelain crucible is placed on a brass plate and covered with a flask from which the bottom has been removed. This serves to keep away currents of air and supports the thermometer, which passes through a cork in the neck, so that the bulb is immersed in the mercury. In the first examination of a substance the temperature is raised somewhat rapidly, the upper disc being removed by forceps and exchanged for a fresh disc at every rise of  $20^{\circ}$ , until the substance disappears. A second determination is conducted more slowly and the discs more frequently changed, while in conducting the third determination the heat is raised very cautiously, and the discs changed every half degree when the previously ascertained subliming point is nearly reached. Blyth defines the subliming point as the lowest temperature, which, if maintained for 60 seconds, allows of the formation of the most minute dots, films, or crystals which can be observed by a microscopic power of  $\frac{1}{4}$  in.

The great majority of subliming points given in this work have not been determined in the above exact manner.

**Boiling Point.**—In making this determination care must be taken that the thermometer bulb is slightly above the surface of the liquid, which should be caused to boil rapidly. The liquid may be contained in a simple test-tube fitted with a cork carrying the thermometer and a short open tube for the escape of the vapour. A small tubulated flask or retort may be substituted for the test-tube. When the quantity of the liquid at disposal is only small, the test-tube should be thin and immersed in a flask half filled with glycerol, paraffin, sulphuric acid, or other suitable liquid. On heating the contents of the flask, the thermometer fitted to the test-tube continues to rise till the b. p. of the liquid is attained, when it remains stationary till

<sup>1</sup> For slightly impure substances the Commercial American practice is to take the melting "range"; for details see U. S. P. ix, p. 596.—*Amer. Ed.*

the latter has evaporated. A very small quantity of liquid suffices for the determination of the b. p. in this manner.

For general purposes the apparatus of Berthelot is convenient. Figure 6, from Traube's *Physico-chemical Methods*, shows its construction. The thermometer is enclosed in an outer tube, so that the portion of the scale to which the mercury rises is immersed in the vapour. If this be not done, a correction must be applied for the error produced by the cooling of the thermometer tube. The bulb of the thermometer does not reach into the liquid. A few fragments of pumice-stone or broken clay pipestem will prevent bumping. The exit-tube at the lower end of the wide tube connects with a condenser. The barometric pressure must always be noted and correction made for the departure from the standard pressure, 760 mm., by the following formula:

$$B = B^1 + 0.0375 (760 - P); \text{ in which}$$

$B$  is the b. p. at normal pressure,

$B^1$  the observed b. p.,

$P$  the observed pressure in mm.

**Distillation** does not need detailed description. For cooling the vapour some form of Liebig's condenser is commonly employed. A useful modification, by which distillation can be made at once to succeed digestion, without rearrangement of the apparatus, has been described by W. A. Shenstone (*Trans.*, 1888, **53**, 123).

Recently several types of double surface condensers have been devised with the object of rendering the condensing action more efficient, so that shorter condensers may be employed than is possible with the old Liebig type. The Cribb condenser is an instance of such a form. Probably the most convenient for all-round work is Davies' condenser, made by Messrs. Gallenkamp, of London.<sup>1</sup>

**Fractional distillation** is an analytical process closely related to the determination of the boiling and subliming points of organic substances; by repeating the process of distillation and collecting apart the fractions which distil at every small increase of temperature, very perfect separation may sometimes be effected.

<sup>1</sup> For latest methods see Menzies, *J. Amer. Chem. Soc.*, 1921, **43**, 2309, 2314.—*Amer. Ed.*

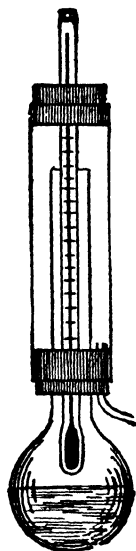


FIG. 6.

When only a small quantity of a complex liquid is submitted to fractional distillation, it is better to keep the bulb of the thermometer wholly immersed in the liquid, as the error liable to be caused by this arrangement is far less than ensues, especially towards the end of the distillation, from the temperature of the residual liquid rising more rapidly than the thermometer can acquire the temperature of the vapour.

In conducting a fractional distillation, it is desirable to operate on a known weight or volume of the substance, and to note the proportion of the whole which passes over at every few degrees of rise in the temperature of the distilling liquid. Details of the precautions which should be taken to ensure constant results will be found in the section treating of the assay of commercial benzols (see Vol. III).

Fractional distillation is a process of the utmost value for effecting the proximate analysis of a mixture of organic substances of different b. p. Speaking generally, the first portions which distil will contain the greater part of the more volatile constituents of a complex fluid, but the composition of the distillate at various stages of the process depends on many circumstances besides the b. p. and relative proportions of the constituents of the mixture operated upon.

Wanklyn showed that the proportion in which the constituents of a mixture pass over depends not only on their relative abundance in the mixture undergoing distillation, and on their respective vapour-tensions at the temperature of ebullition, but also on their mutual adhesion and on the densities of their vapours. He found that, when a mixture of equal weights of two liquids of different b. p. was distilled, the quantity of each constituent in the distillate was proportional to the product of its vapour-density and vapour-tension at the temperature of ebullition of the fraction. Hence, in certain cases, the less volatile of two substances may pass over most rapidly—that is, be found in largest quantity in the first fraction of the distillate. This is true of a mixture of methyl alcohol (boiling at  $65.2^{\circ}$ ) and ethyl iodide (boiling at  $72^{\circ}$ ). If the vapour-tensions and vapour-densities, of the two liquids are inversely proportional, the mixture will distil unchanged.

M. C. Lea found that, on distilling a mixture of ethylamine, diethylamine, and triethylamine hydrochlorides with sodium hydroxide, the whole of the last amine, which is the least volatile of the three, was contained in the first portions of the distillate, provided

that its proportion was not excessive. A similar anomaly is observed on distilling solutions of acetic acid and its homologues.

Sometimes anomalous results ensue, owing to the fact that the tension of the mixed vapours is never equal to the sum of the tensions of the individual vapours. Berthelot found that when a mixture of 90.9 parts of carbon disulphide (boiling at  $46.6^{\circ}$ ), with 9.1 of alcohol (boiling at  $98.4^{\circ}$ ), was distilled, it behaved as a homogeneous liquid. If either of the constituents was present in excess of the above proportion, it remained in the retort in an unmixed condition after the definite mixture had distilled over. Thorpe, again, found that a mixture of equal volumes of methyl alcohol and carbon tetrachloride distilled at a temperature nearly  $10^{\circ}$  lower than that of the b. p. of the most volatile constituent, and the carbon tetrachloride, which has the higher b. p., occurred most largely in the first fractions of the distillate.

In cases where two immiscible liquids are distilled together, the b. p. is the temperature at which the sum of the vapour-tensions is equal to the atmospheric pressure. Thus benzene and water distil together at  $69.1^{\circ}$ , at which temperature benzene vapour has a tension of 533.7 mm., and steam 224.2 mm., the sum of the two being 757.9 mm.

The subject of distillation has been exhaustively studied by Prof. Sydney Young (see especially *Trans.*, 1895, 679; 1902, 707, 768; 1903, 68, 77; Young and Fortey, *Trans.*, 1902, 717, 739 and 752; 1903, 45). See his treatise on *Fractional Distillation* (Macmillan & Co., Ltd., 1922).

From a consideration of the foregoing facts it will be evident that a complete separation of a complex liquid into its constituents is never possible by a single fractional distillation, and that in certain cases it is impossible even on repeating the operation a very great number of times.

A great improvement in the practice of fractional distillation was made by Warren, who, in his researches on American petroleum, employed a Liebig's condenser inclined towards the distilling flask, and kept at such a temperature as to cause condensation of the less volatile constituents of the mixed vapour, while those of lower b. p. passed on to a condenser kept cool in the usual way, and inclined in a direction opposite to the first.

Many arrangements have been devised by which the vapour of the

distilling liquid is partially condensed and succeeding portions are caused to be washed with the liquid produced, which periodically runs back into the distilling flask.

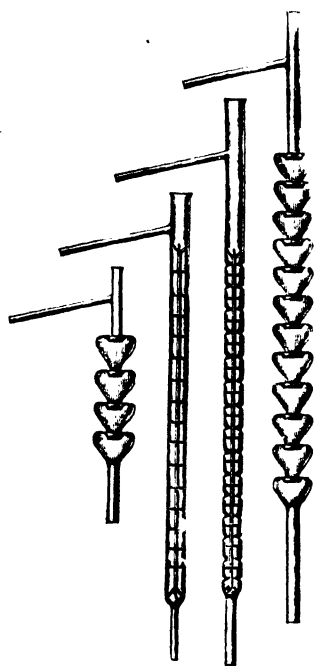


FIG. 7.

A very useful arrangement of this kind is that of Le Bel and Henninger (Fig. 7) which consists of a number of bulbs, ranging from 2 to 12, blown upon a tube, which is fitted by means of a cork into the mouth of the flask containing the liquid to be distilled. The upper end of the tube is furnished with an inclined side-tube, which can be fitted by a cork to a condenser, and with an orifice through which a thermometer can be passed, so as to observe the temperature of the vapour which passes over. Each of the bulbs is connected with the one below by means of a small side-tube. In the constriction of each bulb is placed a small cup of platinum or copper gauze, of the size and shape of a small thimble. These cups are made by folding the gauze over the end of a stout glass rod. The ascending vapour condenses in

the cups, and thus serves to wash the vapour subsequently formed, as it bubbles through. When the liquid rises to a certain height in each bulb it runs off by the side-tube, and ultimately finds its way back to the distilling flask, the flame under which is so regulated as to keep all the cups full, and cause the distillate to fall from the end of the tube in separate drops. In an improved form of dephlegmator, devised by Glynsky (Fig. 9), the wire gauze is replaced by hollow balls of glass, introduced into the bulbs during manufacture.



FIG. 8.



FIG. 9.

Hempel (*Zeit. Anal. Chem.*, 1881, 20, 502) substituted for the more complex arrangement a long wide glass tube, arranged vertically and filled with solid glass beads. By this contrivance he obtained alcohol of 95% by slowly distilling spirit of 18%.

For a comparative study of the efficiency of different types of still-head see a paper by S. Young (*Trans.*, 1899, 75, 679), in which new forms are also described. The types of still-head (made by J. J. Griffin & Sons, London) shown in Fig. 7, are very efficient. When a substance decomposes on boiling under ordinary pressure, it can often be purified by distillation under reduced pressure. For methods see Gattermann's *Practical Methods of Organic Chemistry*, and, in greater detail, Lassar-Cohn's *Arbeitsmethoden f. organischchemische Laboratorien*.

## OPTICAL PROPERTIES

**Refraction and Dispersion.**—The refractive index of a liquid is often a valuable means of identification. The most convenient instrument for accurately measuring refractive indices is Pulfrich's refractometer made by the Zeiss company. For detailed description and instructions for use see Findlay's *Practical Physical Chemistry*. Special types of instrument for measuring the refractive index of butter fat, milk fat, or beer are manufactured by the firm of Zeiss.

## REFRACTOMETERS

As the refractometer is most widely used in analysis in dealing with fats and oils, the description of the different types of this instrument will be included in Volume II. A few of the principal types of refractometer are shown below.

Figure 10 shows the Pulfrich instrument, made by the Zeiss company, and used for measurements of the refractive index of liquids and solutions. It is fully described in a pamphlet issued by the makers and in most works on elementary physico-chemical measurements, *e. g.* Findlay's "*Practical Physical Chemistry*" (Longmans). Figure 11 shows the Abbé refractometer which is used for liquids having a refractive index between  $= 1.3$  and  $1.7$ .

In this instrument the liquid to be tested is placed between two similar prisms which must be of greater refractive index than the

sample. When light meets the surface separating the lower prism from the liquid, it is totally reflected if the angle of incidence is greater than the critical angle. Hence, if the double prism be viewed through a telescope the field will be partly dark and partly bright. The telescope is attached to a sector bearing a scale, while the double prism is connected with an arm which carries an index moving over the divided scale. By rotating the prisms the critical line dividing the field of view can be brought to coincidence with the centre of the cross-webs in the eye-piece. The reading on the scale then gives the index of refraction without any calculation.

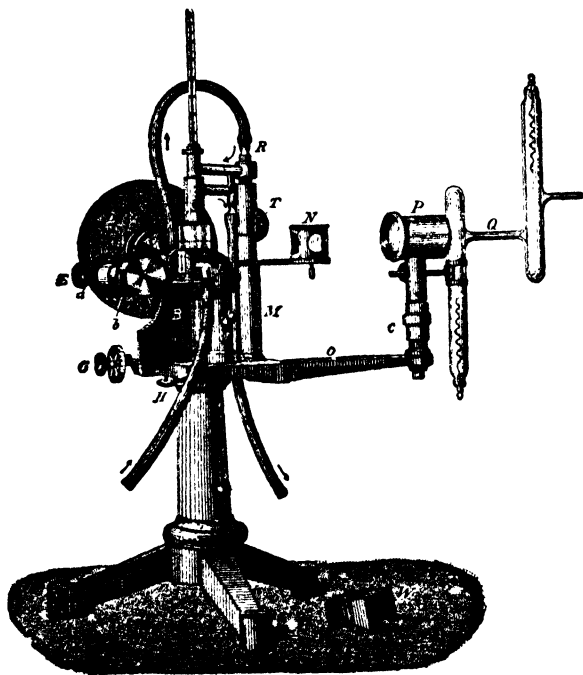


FIG. 10.

If the light used is not monochromatic the position of the critical line in the field will vary for each component colour. There will thus be a coloured fringe dividing the two portions. In order to annul this colour disturbance a compensator is introduced which consists of two direct-vision prisms of equal dispersion, and can be rotated by means of a screw in opposite directions round an axis parallel

to the line of vision. The reading on the divided head of the compensator when the colour fringe is neutralised gives the dispersion of the liquid for the particular light used.

Figure 11 shows the form of instrument used when it is required to maintain the test liquid at a certain temperature. The prisms are mounted in metal boxes, through which a stream of water to the

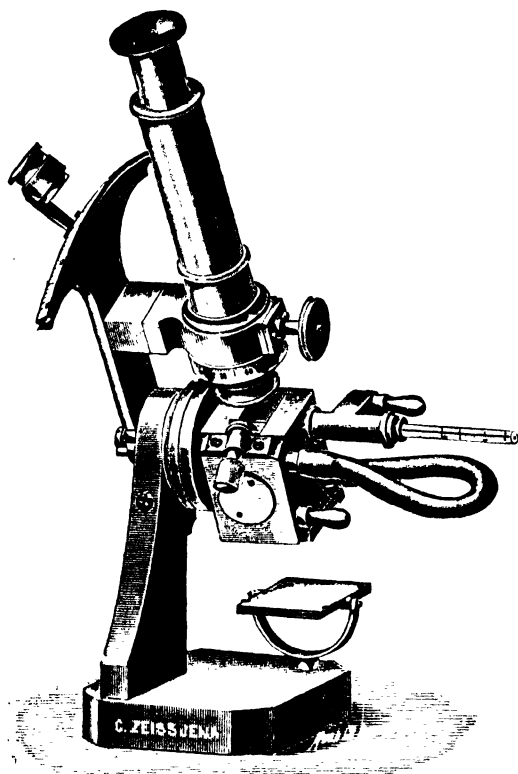


FIG. 11.

requisite temperature is passed from a convenient source. The Abbé refractometer with non-heating prisms (Fig. 12) is used in measuring refractive indices of solid bodies (*e. g.*, crystals) and of viscous plastic substances. The measurements are made by means of either reflected or grazing incident light. The Zeiss company supplies on application a pamphlet describing in detail the construction and use of the Abbé refractometers; a special pamphlet giving a

complete bibliography of the technical application of refractometers is also issued by the same firm.

As the *Immersion Refractometer* of Zeiss is now very widely used in commercial organic analysis (for example, in the estimation of alcohol in beers by Ackermann's method; in the examination of products containing grape or cane sugar; in the analysis of blood and

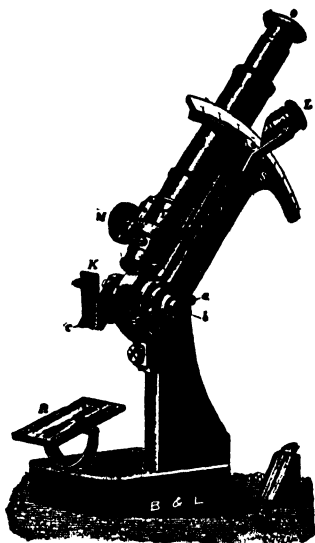


FIG. 12.

exudations), a short account may be given of this instrument, which is illustrated in Fig. 13. The method of measurement is founded on the observation of the border line of total reflection in a telescope as in the refractometers mentioned above, but the manipulation is much more simple than with these instruments. The prism (Fig. 14) at the lower end of the refractometer held vertically is simply immersed in the solution contained in a well-filled beaker.

The prism body is cylindrical in shape (so that awkward corners and indentations difficult to keep clean do not exist) and only glass parts are immersed, so that the instrument can be used for acid solutions (for example, acetic acid). In using the instrument care must be taken that day- or lamp-light passes into the fluid parallel to the oblique prism surface, as indicated in Fig. 15, which shows in

section an old type of the instrument now superseded by that of Fig. 13. In the new type of dipping instrument, light is reflected from the mirror below the trough.

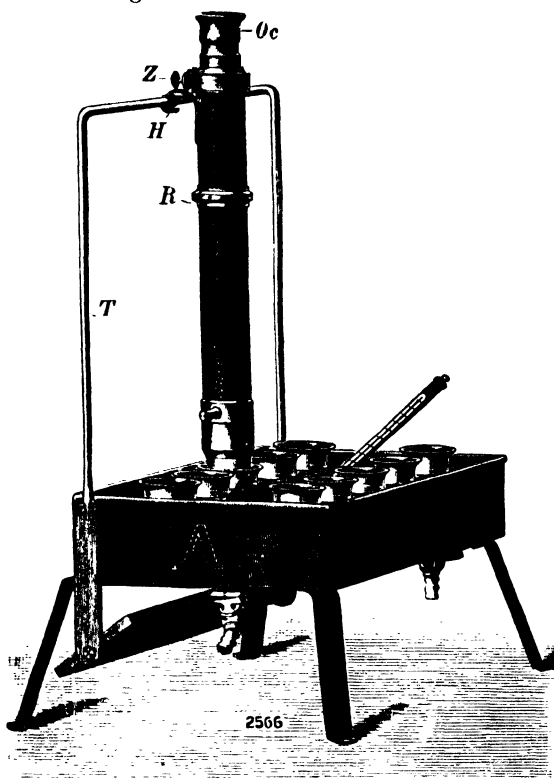


FIG. 13.

The lower end of the refractometer is immersed in the middlemost of the 5 beakers of the front row. The rectangular mirror fitted under the trough reflects the light of the bright sky through a glass plate upwards into the beaker and through the fluid into the refractometer. The latter hangs by its hook *H* upon the wire frame *T*. Observations are made from above by means of the ocular *Oc*. The border line of total reflection is achromatised by turning the milled ring *R*. The micrometer screw *Z* gives one-tenth scale divisions.

Conforming to the new process of observation, the instrument consists essentially of the following parts.

A *prism P* of hard glass, with a refracting angle of about  $63^\circ$ .

A *telescope*, rigidly connected with the prism, formed by the objective *O* and eye-piece *Oc*, with the Scale *Sc* and Micrometer Screw *Z* in Fig. 13.

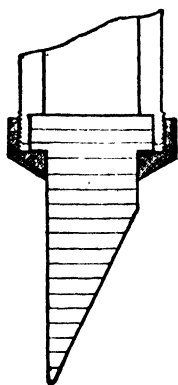


FIG. 14.

A compensator *A*, placed between prism *P* and objective *O*, which can be rotated about the axis of the telescope by means of the milled ring *R*.

The border line, which separates the bright part of the field from the dark, is, on account of the difference in dispersion between glass and fluids, generally fringed with colour and quite unsuitable for an exact reading. On rotating the compensator by means of the milled ring *R* the colour disappears and the separating line between dark and light becomes quite sharp and colourless. The position of this sharp line relative to the scale is the measure of the refractive index of the fluid. Table IV gives the value of the refractive index corresponding with

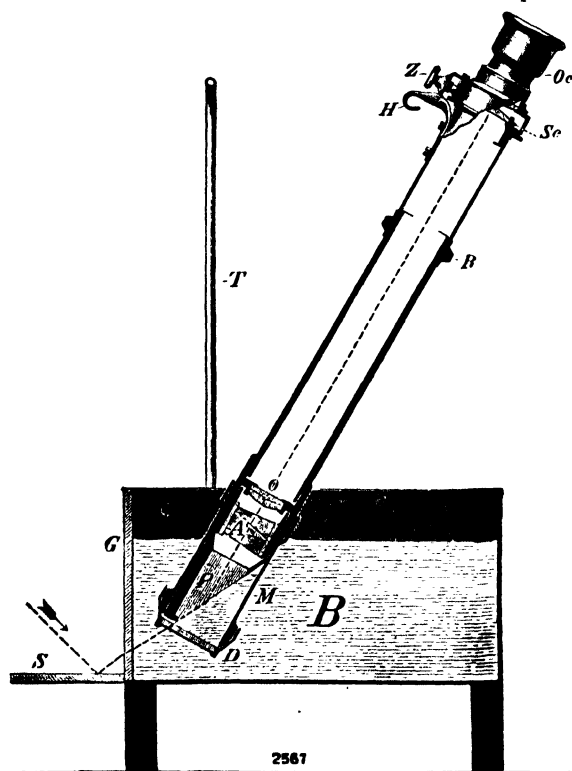


FIG. 15.

each scale division. The scale divisions are read directly and intermediate positions calculated in decimals of a division by means of the micrometer screw  $Z$ , the scale being slid across the border line until the scale division previously noted stands contiguous with it. The division on the micrometer drum then shows the decimal of the scale index.

The immersion refractometer has a range of measurement between a refractive index  $n_D = 1.325$  (sea-water in the tropics) and  $n_D = 1.366$  (alcohol). Within this range fall the refractive indices of aqueous solutions of salts, acids, sugars, and of liquids, such as beer and wine. In spite of its simplicity, this refractometer excels in the accuracy of its readings all other kinds of refractometer, with the exception of interference refractometers. It is of course necessary that observations should be made at a known temperature,  $17.5^\circ$  being the temperature corresponding with the values in the table.

TABLE IV

Table for the Calculation of the Scale Divisions of the Immersion Refractometer in Refractive Indices  $n_D$  and *vice versa*

Scale divisions	$n_D = 1.3$			Scale divisions	$n_D = 1.3$		
-5	25 39			50	46 50		
-4	25 78			51	40 87		
-3	26 18			52	47 24		
-2	26 57			53	47 61		
-1	26 96			54	47 98		
0	27 36	40		55	48 36	37	
1	27 75			56	48 73		
2	28 14	1	4.0	57	49 10	1	3.7
3	28 54	2	8.0	58	49 47	2	7.4
4	28 93	3	12.0	59	49 84	3	11.1
		4	16.0			4	14.8
5	29 32	5	20.0	60	50 21	5	18.5
6	29 71	6	24.0	61	50 58	6	22.2
7	30 10	7	28.0	62	50 95	7	25.9
8	30 49	8	32.0	63	51 32	8	29.6
9	30 87	9	36.0	64	51 69	9	33.3
10	31 26			65	52 05		
11	31 65			66	52 42		
12	32 04			67	52 79		
13	32 42			68	53 16		
14	32 81			69	53 52		
15	33 20			70	53 88		
16	33 58	39		71	54 25	36	
17	33 97			72	54 61		
18	34 35			73	54 97		
19	34 74			74	55 33		
20	35 13	1	3.0	75	55 69	1	3.6
21	35 51	2	7.8	76	56 06	2	7.2
		3	11.7			3	10.8

TABLE IV.—*Continued*

Scale divisions	$n_D = 1.3$			Scale divisions	$n_D = 1.3$		
22	35.90	4	15.6	77	56.42	4	14.4
23	36.28	5	19.5	78	56.78	5	18.0
24	36.67	6	23.4	79	57.14	6	21.6
		7	27.3			7	25.2
25	37.05	8	31.2	80	57.50	8	28.8
26	37.43	9	35.1	81	57.86	9	32.4
27	37.81			82	58.22		
28	38.20			83	58.58		
29	38.58			84	58.94		
30	38.96			85	59.30		
31	39.34			86	59.66		
32	39.72			87	60.02		
33	40.10			88	60.38		
34	40.48			89	60.74		
35	40.86			90	61.09		
36	41.24			91	61.45		
37	41.62			92	61.81		
38	41.99			93	62.17		
39	42.37			94	62.52		
40	42.75			95	62.87		
41	43.13			96	63.23		
42	43.50			97	63.59		
43	43.88			98	63.94		
44	44.26			99	64.29		
45	44.63			100	64.64		
46	45.00			101	65.00		
47	45.37			102	65.35		
48	45.75			103	65.70		
49	46.12			104	66.05		
50	46.50			105	66.40		

**Example:** Scale division 3.1 corresponds to the Refractive Index  $n_D = 1.32854 + 0.000039 = 1.32858$ .

A convenient type of water heater and water-pressure regulator by means of which the trough *A* is maintained at this temperature, is shown in Fig. 16, and is described in detail in pamphlets issued by the Zeiss company. The arrangement devised by Lowry for supplying water to polarimeter tubes at a definite temperature can also be used (page 56). The description of the Zeiss heater is as follows:

The spiral heater, about 3.5 metres in length, of stout copper, is enclosed in the space between two telescoping metal cylinders. The inner cylinder is provided with a copper bottom, through which the heated air generated by a bunsen burner, petroleum or spirit lamp is evenly distributed and conducted to the copper pipe.

The top of the spiral heater is connected with the tap *C* of the heating trough *A* by a short length of tightly stretched tubing, which should incline upwards in the direction of *C*. In this way the accumulation of air bubbles, which would otherwise obstruct the uniform

flow of water, is checked. By means of the vessel *B* the water is drained off by a glass or metal tube and led to a sink. When desired,

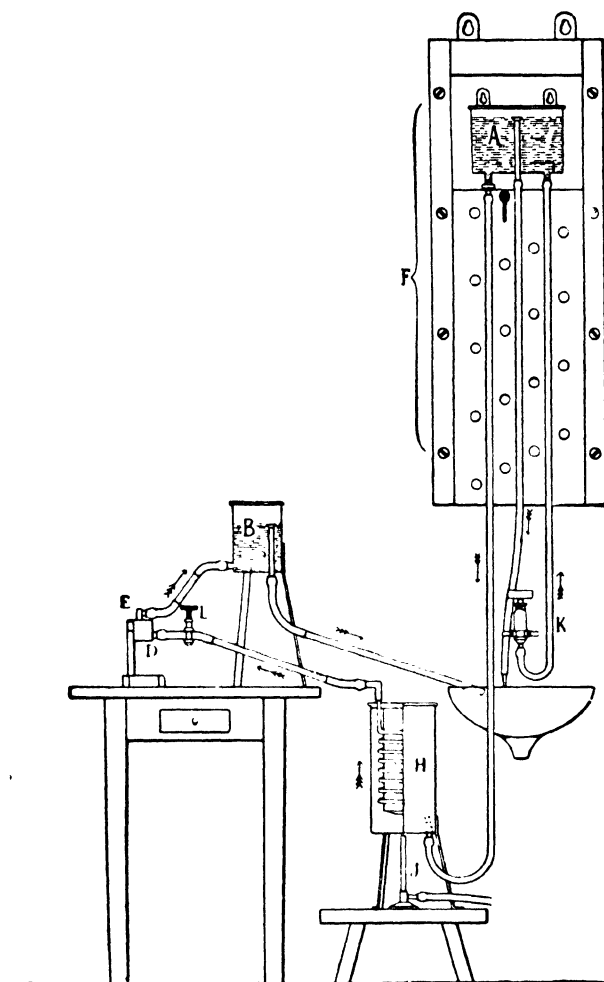


FIG. 16.

the heating apparatus can be thrown out of action by turning off the gas, turning the cock at *C* and drawing off the water.

It is generally best not to have a too sluggish flow of water, and to obtain a certain approximate temperature first by appropriate manipulation of the source of heat; the temperature is finally adjusted to the exact degree required, by varying the difference in elevation between the cistern *A* of the water-pressure regulator and the heating trough *A*.

The difference in elevation between the cistern *A* and the heating trough may be varied in two ways: cistern *A* is either suspended from a cord running on a roller, the free end of which is made fast as with roller blinds, or it is hung on two hooks driven into a board. The latter can be made to slide up and down in a frame formed of a board about 1 metre in length and fitted with two strips placed lengthwise at the sides, the whole being fastened against a wall. Holes are bored through this board arranged in zig-zag lines and each about 1 cm. above the next lower one, the board with cistern *A* being kept at the desired height by passing a peg through one of the holes. It is an easy matter to find by trial the number of holes by which the board requires moving in order to cause the temperature to vary by 1°.

*Manipulation of the Immersion Refractometer.*—It is first necessary to see that the instrument is properly adjusted. For this purpose the heating trough *A* (Fig. 13) is placed with its long side parallel to the window and the mirror turned towards a bright sky. The trough is then half-filled with water and a beaker filled with distilled water is placed in one of the five holes in the front row immediately above the mirror. Finally the refractometer is hung by the hook *H* upon the wire frame so that the prism is completely submerged in the water contained in the beaker.

The whole apparatus is now allowed to stand for 10 minutes or so to bring everything to the same temperature. When the distilled water has exactly taken the temperature of the bath, the eyepiece is focussed on the divisions of the scale by turning the milled head of the eye-piece until the lines and numbers are seen quite distinctly, and the mirror adjusted so that the light of the bright sky is seen directed *through the beaker*. The upper part of the field, from  $-5$  to about 15 appears bright and is separated from the lower dark part by a sharp line of demarcation, if the index on the ring of the compensator stands at 5.

TABLE V

The correctly adjusted refractometer should show, for distilled water at

Temperature.....	10° C.	11	12	13	14	15	16	17	17.5	18	19° C.
The Scale Division	16.3	16.15	16.0	15.85	15.7	15.5	15.3	15.1	15.0	14.9	14.7
Temperature.....	20° C.	21	22	23	24	25	26	27	28	29	30° C.
The Scale Division	14.5	14.25	14.0	13.75	13.5	13.25	13.0	12.7	12.4	12.1	11.8

The reading is taken as already explained and the temperature of the distilled water noted. Reference to Table V<sup>1</sup> will show if the Refractometer be correctly adjusted.

By means of this table it is possible to test the adjustment of the refractometer without having first to adjust the bath to the normal temperature of 17.5°. Should the average of further careful readings deviate from that contained in Table V, the following should be resorted to:

The eyepiece end of the refractometer, hanging on the wire frame, is grasped from behind with the thumb and fore-finger of the left hand, the micrometer drum set to 10 and the steel point enclosed in the case of the apparatus, inserted into one of the holes of the nickelled cross-holed screw, lying on the inner side of the micrometer drum. The point is then turned *anti-clockwise* (as seen from the rear) whereupon the nickelled milled nut, which governs the micrometer, becomes loosened. The temperature of the distilled water in the beaker is again read to ensure that it has remained constant and then Table V is consulted to find the "*adjusting number*" properly belonging to the temperature indicated. By turning the point the border line is brought exactly on the integer scale division belonging to the adjusting number, and the still loose micrometer drum is turned so that the index corresponds with the decimal portion of the adjusting number. The drum is now held firmly with the thumb and fore-finger of the left hand, while the *nut* is again

<sup>1</sup> "Über quantitative Bestimmungen wässriger Lösungen mit dem Zeiss'schen Eintauch-Refraktometer." By Medizinalassessor, Dr. B. Wagner, Jena, 1903 (*Diss.*), p. 14.

screwed up tight by the right hand, care being taken that the drum does not wander off the index. Finally the new adjustment is tested by repeated readings. After the instrument has been properly adjusted, measurements can be made in the manner already described, using a beaker full of the liquid to be examined. When only small quantities of the liquid are available (as, for example, in dealing with blood serum) or the solution is too deeply coloured, as in the case of dark beer or molasses, an *auxiliary prism* is used, the face of which is laid on the polished elliptical face of the refractometer prism. The liquid to be examined is applied between the two prism faces, which are then locked into position by a suitable cover. Details for the use of the auxiliary prism are supplied by the makers of the instrument.

When a volatile liquid or solution which would quickly evaporate has to be examined, a metal beaker *M* in Fig. 15, supplied with the instrument, is used; it is clamped to the prism of the refractometer by means of the bayonet-joint, and, while the refractometer is held with the prism pointing upwards, it is filled with the liquid to be examined. The cap *D* is then carefully fitted and locked, and the observation made by hanging the refractometer in the wire frame of the trough *A*, so that the metal beaker is submerged in the bath. If trough *B* is used (older type of instrument) the refractometer is inclined as shown in Fig. 15.

## SPECTROMETERS AND SPECTROGRAPHS

**The absorption-spectrum** of an organic substance occasionally furnishes information not to be obtained in any other way, and in the examination of blood-stains, dye materials, and other coloured substances is often of great utility.

**Spectrometers and Spectrographs.**—Probably the most convenient spectrometer for use in technical analysis, especially in the observation of absorption spectra, is the Hilger wave-length spectrometer of the constant deviation type, made by A. Hilger, London, and shown in Fig. 17.

The prism is of a special form (Fig. 18) and may be considered as built up of two  $30^\circ$  prisms and one right-angled prism from which the light is internally reflected. Usually the prism is made in one piece, but with very highly refractive glass it is built up of the separate prisms.

The telescope and collimator are always at right angles, being fixed in this position, the different parts of the spectroscopic field being brought across the pointer in the eyepiece of the observation

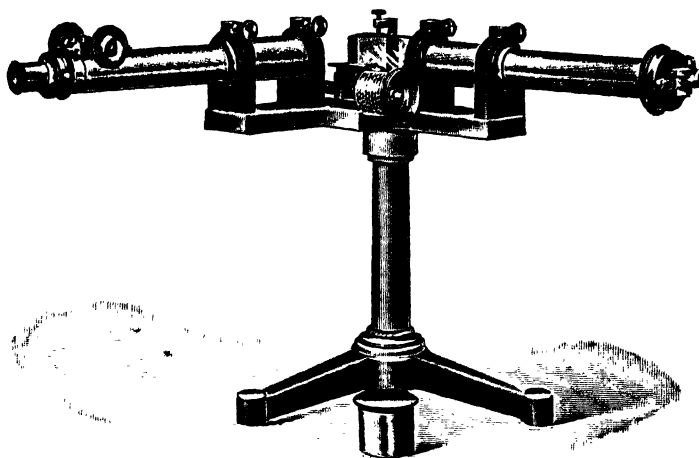


FIG. 17.

telescope (used instead of cross-wires) by rotating the prism, this being effected by means of a fine steel screw, the point of which

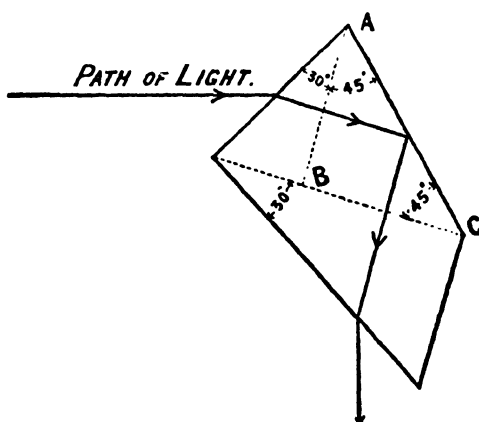


FIG. 18.

presses against a projecting arm on the prism-table. To the screw is fixed a helical drum (see Fig. 19) on which the wave-length of the

line under observation and coinciding with the cross-wires in the telescope is read off directly; the wave length being indicated by the index, which runs in a helical slot.

With this instrument, which reads to within 2 Ångström units and in which a single high dispersion prism separates the two  $D$  sodium lines by an apparent distance of about  $\frac{1}{32}$  in., the accurate observation of spectra is enormously simplified. In using the instrument the prism is first fixed in position by reference to one or two lines in the spectrum. For this purpose the drum is first rotated until the

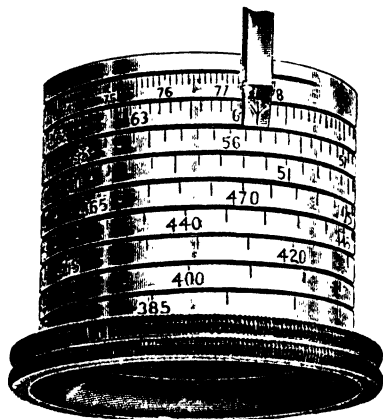


FIG. 19.

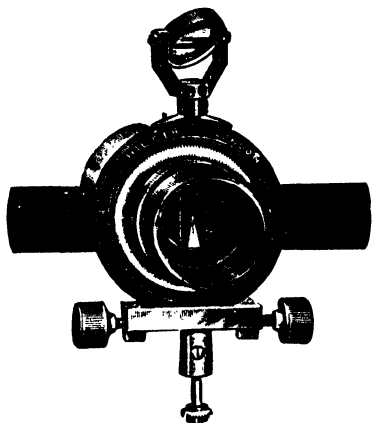


FIG. 20.

pointer corresponds with the wave length of one of the sodium lines ( $D_1$  or  $D_2$ ); a sodium flame is then put before the collimator, the slit adjusted and the prism rotated carefully by hand, until the sodium line chosen corresponds exactly with the bright pointer in the eye-piece. To simplify the adjustment, the bright pointer itself (shown in Fig. 20) can be moved laterally by the two milled-head screws below; the metal pointer in the eye-piece is ground exceedingly fine, brightly polished and illuminated from above by the small mirror (Fig. 20). When the position of the prism has been found (an operation which occupies a few minutes only), it is clamped in position by the top screw; its outline should, when the instrument is first used, be marked out by pencil on the base plate. On all subsequent occasions the prism is placed approximately in position by means of this outline and can then be accurately adjusted in a few moments. When the position of the prism has been fixed by

reference to the sodium line the wave-length scale gives the position of every other line in the spectrum. The accuracy of the calibration and of the adjustment of the prism can be tested by reference

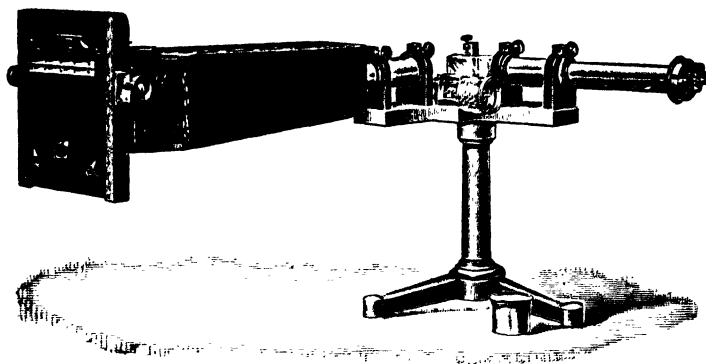


FIG. 21

to any line in the extreme blue; for instance, by means of the blue caesium line. For this purpose a trace of a caesium salt is heated on a platinum wire in a bunsen flame.

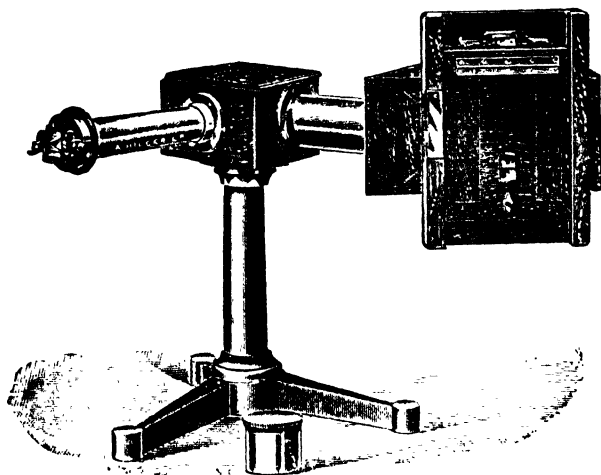


FIG. 22.

To observe an absorption spectrum, it is only necessary to place a luminous flame—for example, a Welsbach-burner—before the colli

mator and to interpose a trough (of a suitable thickness) filled with the liquid under observation. The absorption spectrum is then produced and the wave lengths of the absorption lines or bands can be read off directly on the scale by turning the graduated drum until

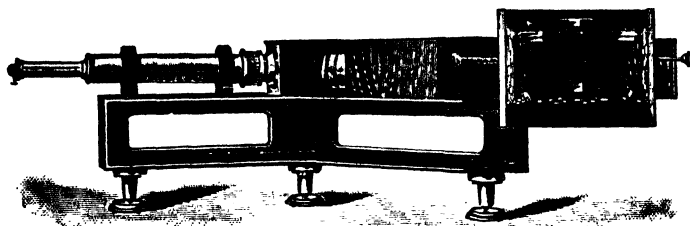


FIG. 23.

the absorption bands correspond with the bright pointer in the eyepiece. The two shutters in the eyepiece, which can be moved laterally, are of great service in observing faint lines, as they can be

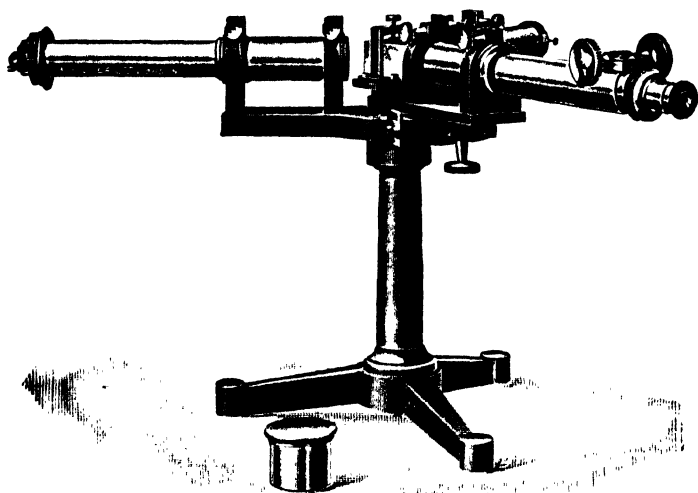


FIG. 24.

shifted from either side so as to cover any desired part of the field and thus prevent the eye from becoming fatigued by the glare in the rest of the field.

A useful accessory to the above spectrometer is the camera shown in Fig. 21 which is used as a spectrograph.

This instrument is of service in recording photographs of absorption spectra.

When absorption occurs in the ultra-violet region of the spectrum it is necessary, in order to obtain photographs of the absorption spectrum, that the lenses and prism of the spectrograph be constructed of special glass (ultra-violet glass) which is transparent to the ultra-violet rays; Fig. 22 shows a spectrograph which is suitable for use in obtaining photographs, for example, of the absorption bands of blood.

Quartz lenses and prisms have to be used when the extreme ultra-violet region—that is, the region of smallest wave length—has to be examined. Figure 23 shows a spectrograph with quartz train made by Messrs. Hilger.

A much cheaper instrument than any of the foregoing is Hilger's wave-length spectrometer (Fig. 24) of the photographic scale type; measurements can be made with it even more rapidly than with the drum-reading spectrometer, but it is not quite so accurate. The readings are, however, correct to within about 10 Ångström units.

The photographic scale is mounted on a tube (with collimating lens), and the light from the scale is reflected from the surface of the prism. A reflected image of the scale is thus seen in the telescope in juxtaposition to the spectrum as shown in Fig. 25 which shows a portion of the complex absorption spectrum of nitric oxide. The dark bands are, of course, not sharply defined in this spectrum. The definition of the scale is very much finer than is shown in the print. The print is the exact size of the real image formed by the telescope object glass. To get an idea of the size of field in the instrument, therefore, the print should be looked at with an eyepiece.

The collimator, prism and photographic scale mount are fixed to a rigid cast-iron base, the telescope alone rotating to pass through the spectrum. To this instrument a camera can also be attached so as to produce photographic records of the spectra under examination.

The Hüfner spectrophotometer (Fig. 26) made by Hilger is designed for the accurate measurement of absorption of liquids for light of any desired wave length. It has found a useful application in the determination of the densities of photographic plates and,

more recently, in the quantitative estimation of minute quantities of nitrogen peroxide, such as are produced by the decomposition of nitro-explosives (see Robertson and Napper, *Trans. Chem. Soc.*, 1907, 91, 761 and 764). It may here be mentioned that the wavelength spectrometers already described are particularly convenient in carrying out the test for mercury in nitro-explosives, such as cordite. (Compare the article on "Explosives," in Vol. III.)



FIG. 25

The Hüfner spectrophotometer consists of the following essentials: It is desired to compare the intensities of two beams of light, one of which has undergone absorption (by passage, for instance, through a known thickness of a liquid under observation). In the path of the beam which has not undergone absorption is interposed a Nicol prism, which polarises the light perpendicularly.

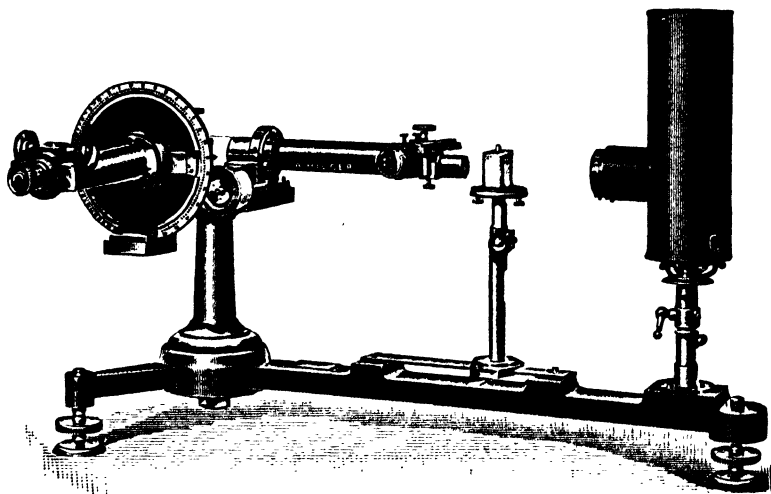


FIG. 26.

The two beams of light are then thrown on the slit of the spectroscopic portion of the apparatus, being brought into close juxtaposition with a sharp dividing line by a prism of special design. The light after passing through the slit undergoes collimation, and is spread into a spectrum by a prism, and after passing through a second Nicol prism is brought to a focus, and observed by an eye-

piece. Two spectra are then seen one above the other with a very sharp dividing line between; the one being an absorption spectrum of the liquid substance under observation, the other spectrum being reducible by rotation of the second Nicol prism to any desired intensity. The intensity of this latter spectrum can be simply deduced from the rotation of the second Nicol, and thus, by exact matching of any desired part of the two spectra, an exceedingly accurate measurement of the amount of absorption of the observed material can be obtained. One can pass through the spectrum by a screw motion, with a large drum-head on which the part of the spectrum under observation is marked in wave lengths. Owing to the special form of prism used, the telescope is rigidly fixed as in the constant deviation spectrometer.

The rotation of the second Nicol is read off by a vernier. The eye-piece has two shutters which can be pushed in from right and left, by means of which any part of the spectrum can be isolated (see page 36, Fig. 20).

The following works deal with the observation of absorption spectra; Formánek, *Die qualitative Spectralanalyse* (R. Mückenberger, Berlin); E. C. C. Baly, *Spectroscopy* (Longmans). The most exhaustive treatment of the subject is contained in Kayser's comprehensive "*Handbuch der Spectroscopie*."

**Microspectroscope.**—For observing the absorption-spectra of organic substances a pocket spectroscope will often suffice, but it is far better to employ a microspectroscope, furnished with a proper comparison stage and reflecting prism, so as to allow of the spectrum of the colouring matter under examination being viewed in juxtaposition with the spectra of standard specimens of known origin.

**Fluorescence** of organic bodies is a qualitative character often of much value. It is absolutely necessary that the liquid to be observed should be *perfectly clear*, as the presence of minute suspended particles may lead to fallacious conclusions.

As a rule, the phenomenon of fluorescence may be observed by filling a small test-tube with the fluorescent liquid, holding it in a vertical position before a window, and observing the liquid from above against a dark background. Another plan is to make a thick streak of the liquid on a piece of polished jet or black marble, or on a glass plate smoked at the back, and to place the streaked surface in front of, and at right angles to, a well-lighted window. Either

of these methods is superior to the polished tin plate sometimes recommended. The background should be black, not white.

In some cases, the following method of observing fluorescence may be advantageously employed. A cell is made by cementing a piece of barometer-tube about  $\frac{3}{4}$  in. in length, and having an internal diameter of  $\frac{1}{6}$  in., to a glass microscope-slide, by means of black sealing-wax. The open end of the cell must be well polished. On introducing a clear solution of any fluorescent substance, covering the cell with a piece of thin glass, placing the slide on the stage of a microscope, illuminating the tube at the side by means of strong daylight, and looking down and observing the axis of the cell by a low microscopic power, the liquid will appear more or less turbid and of a colour dependent on the nature of the fluorescent substance in solution. If no fluorescent substance be present, the field will appear perfectly black, as no light is reflected either from the apparatus or the liquid. For a sensitive method of detecting fluorescence see Francesconi and Bargellini, *Atti dei Lincei* 1906, [5] 15, No. 3. When desired, the *spectrum* of the fluorescent light can be observed by the microspectroscope. In some instances the spectrum thus obtained shows remarkable and characteristic bands.

**Double refraction**, as observed under the microscope by means of polarised light, is often of value for the recognition of organic bodies. In addition to the well-known phenomena dependent on crystalline form, many organic substances not actually crystalline exhibit a cross and series of rings when viewed by polarised light. This is notably the case with many of the starches, and furnishes a valuable means for their discrimination. The optical properties of crystals often serve as a means of identification. For the use of the polarising microscope in this connection see Weinschenk, *Anleitung zum Gebrauch des Polarisations-Mikroskops*, Freiburg, 1906.

## POLARIMETERS

**Rotation of the Polarised Ray.**—Organic substances containing a so-called asymmetric carbon atom possess the power of rotating the plane of polarisation of a luminous ray; as this property is exerted even by the *solutions* of optically active substances, the angle through which the rotation occurs often serves for the accurate estimation of certain compounds. The method is much employed in the examination of saccharine substances.

**Construction of Polarimeters.**—In all forms of apparatus for measuring the rotation of the plane of polarisation of a luminous ray, the polariser, or optical means of obtaining a beam of polarised light, consists of a double-refracting prism of calcite. In some cases a double-image prism is used, but in others the extraordinary ray only is employed. The analyser is composed of a Nicol prism, with a suitable eye-piece. On rotating the analyser through  $90^\circ$  the field becomes perfectly dark, but on introducing between the analyser and polariser a tube filled with sugar solution or other optically active liquid the light again passes. With white light the transmitted tint differs with the strength of the solution of sugar and the length of the tube, and rotation of the analyser merely causes an alteration in the colour of the transmitted light, a phenomenon due to the fact that rays of differing refrangibility are rotated unequally (rotatory dispersion). If monochromatic light be employed, a certain angular rotation of the analyser will suffice wholly to extinguish the light from the field of view, and hence, by measuring the angle through which the analyser must be rotated to restore darkness, an estimate of the strength of the interposed liquid in sugar or other active constituent may be obtained. Quartz possesses powerful rotatory action, a plate 3.75 mm. in thickness ( $= 0.148$  in.) rotating the plane of polarisation of the mean yellow ray through 90 degrees. Some specimens of quartz are dextrorotatory, others are lævorotatory. Hence, a double plate composed of equal thicknesses of the two varieties possesses no rotatory power. If a plate composed of semicircles of right- and left-handed quartz, each 3.75 mm. in thickness, is placed between Nicol's prisms, while the principal sections of the latter are parallel, the field assumes a peculiar purple, known as the *transition tint*. The least rotation of the analyser causes one half of the circle to incline to red and the other half to violet, and the interposition of a solution of an optically active substance produces a similar effect, while to restore uniformity of tint necessitates a rotation of the analyser through an angle dependent on the strength and thickness of the polarising liquid used.

Laurent's polarimeter is one of the simplest. One-half of the field of vision is covered by a very thin plate of quartz which causes an alteration in phase of half a wave length and allows light to pass, even when the analyser and polariser (both of which are Nicol's

prisms) are crossed. If the analyser be rotated so as to cause the quartz plate to become dark, light passes through the uncovered half of the field. In a position intermediate between these two the two halves of the field appear equally dark, and this is the zero point of the instrument. The slightest deviation from this neutral position causes one half of the field to appear darker and the other half lighter than before. Hence the change is a double one and the instrument is thus made very sensitive. Monochromatic light must be used.

The Lippich polarimeter, now widely used, is also a half-shadow instrument differing from the Laurent polarimeter in the method adopted for producing the half shadow, a small Nicol prism replacing the quartz plate. Triple-field instruments have also been devised. In these the field is divided vertically into three zones, the central one being a broad band. The optical construction is such that the lateral zones always agree in tint, thus making the contrast with the central portion more marked. In one form of instrument the portions of the field are concentric. It is claimed that this method gives a high degree of delicacy (see below).

The use of monochromatic light, *desirable* in saccharimetry, is *essential* in estimating many substances. This is due to the fact that Biot's law, that the angles of rotation for the different simple colours are proportional to the squares of the indices of refraction and inversely as the squares of the wave-lengths, is true of quartz and saccharine liquids, but does not hold good generally. In all cases, to insure accuracy, not only should monochromatic light be employed, but the liquid under observation be kept at a known temperature.

For monochromatic light, the lamp usually employed is a bunsen burner with a ledge at the top for holding some solid sodium compound. A fused mixture of sodium chloride and phosphate is better than sodium chloride alone. The following is an excellent method for obtaining a steady, strong yellow light: strips of common filter-paper 5 cm. wide and about 50 cm. long are soaked in a strong solution of sodium chloride and thiosulphate, dried, and rolled into a hollow cylinder of such size as to fit firmly on the top of the burner. The cylinder is kept from unrolling by a few turns of fine iron wire. The flame burns at the top of the cylinder, giving for the first few minutes a luminous cone, but soon becoming pure yellow. The

cylinder becomes a friable charred mass, but if not disturbed may be used for some time continuously or at intervals.

An effective method for producing a sodium flame is that devised by Caldwell and Whympster (*Proc. Roy. Soc.*, 1908, **A 81**, 112-117). A Mecker burner is taken off the ordinary base, screwed to a piece of brass tubing and fixed in an ordinary glass bottle by means of a rubber stopper. The gas supply is led into the bottle by a glass tube and passes through a powder consisting of an intimate mixture of finely ground dry sodium carbonate and clean sand. The sand is necessary to prevent the particles of sodium carbonate from caking. So much sodium carbonate is blown up that the whole flame (6 by 1¼ in.) is uniformly coloured an intense yellow. It has about 60 candle power.

H. W. Wiley has pointed out the usefulness of acetylene as a source of light for polarimetric work. By the use of this light he was able to make readings through liquids which were too dark to permit light from ordinary sources to pass. Since acetylene can be readily and safely prepared by self-regulating apparatus, it will doubtless find application in this and in other departments of laboratory work. Landolt recommends an Aron's mercury vapour lamp as a convenient means of obtaining monochromatic light in polarimetry. Lowry has employed (*Proc. Roy. Soc.*, Nov. 19, 1908), the Bastian mercury lamp with great advantage in measuring rotatory dispersion.

Before using the polarimeter the observing tube should be filled with distilled water and placed in position between the polarising and analysing prisms, which are then to be adjusted, so that the latter shall be at the zero point of the scale when there is no optical disturbance of the field. The tube is then filled with the solution to be tested and replaced between the polariser and analyser, when, if it contain an active substance, an optical disturbance will be observed, the extent and direction of which will depend on the amount and nature of the rotating substance under examination. The polarimeter is then adjusted, so that the neutral point is reached, or, in other words, so that the optical disturbance produced by the introduction of the rotating liquid is compensated; the rotation required to produce this effect is then read and recorded. From the circular rotation observed, the specific rotatory power of the substance may be calculated in the manner described in the next paragraph.

Full directions for the preparation of the solution and the practical

management of the polarimeter will be found in the section on the "Sugars."

**Specific Rotatory Power.**—The specific rotatory power of an optically active substance is the angular rotation exerted by it on a ray of polarised light traversing a thickness of 1 decimetre (= 3.937 in.) of the substance.

The *absolute* specific rotatory power of a solid can only be observed by using thick slices of considerable transparency. It is usual to operate on a solution of known concentration, and from the *sensible* or *apparent* specific rotatory power observed, to calculate the *absolute* rotatory power of the solid substance.

The apparent specific rotatory power of a substance in solution  $[a]$  is obtained from the following measurements:

$a$  = The observed angle of rotation in degrees.

$c$  = The concentration of the solution is grm. per 100 c.c.

$L$  = The length of the column of solution in mm.

$$[a] = \frac{10^4 \cdot a}{Lc}.$$

The apparent specific rotatory power of a substance varies greatly with the wave length of the light employed (rotatory dispersion); it is therefore necessary to make the measurements with monochromatic light of one particular wave length and to state the position in the spectrum of the particular ray employed. In practice the rotation of a substance is expressed in two ways. Either it is referred to the  $D$  line of the solar spectrum, the rotation being then expressed by  $[a]_D$ ; or it is referred to the "medium yellow ray" (*jaune moyen*) which is complementary to Biot's transition tint; the rotation being then denoted by  $[a]_j$ . In the former case  $[a]_D$  is measured by a Wild, Mitscherlich, Jellet-Cornu or Laurent instrument and the direct rotation is found in degrees of arc. In the latter case,  $[a]_j$  is measured by means of a neutral-tint or half-shadow polarimeter, such as the Ventzke-Scheibler. The scale divisions in such instruments are arbitrary and have to be converted into angular degrees before the specific rotatory power can be calculated. The readings obtained being based on the rotation of a quartz plate are obtained in terms of the rotation of a quartz plate of definite thickness. Whilst the rotatory dispersion of quartz and cane-sugar solutions are nearly identical, most other substances have a very different rotatory

dispersion from that of quartz. Thus, a quartz compensating instrument can only be used in the comparison of rotatory powers of different substances, when the rotatory dispersion of the substance under examination is known relatively to that of quartz.

The wave length of the "mean yellow" ray being less than that of the  $D$  line, the numerical value of  $[a]_j$  is greater than that of  $[a]_D$ . With a quartz plate 1 mm. thick, Broch found

$$\begin{aligned}[a]_D &= 21.67 \\ [a]_j &= 24.5\end{aligned}$$

Whence:  $[a]_j = 1.1306 [a]_D$  or approximately  $\frac{9}{8} [a]_D$ .

$$[a]_D = 0.8845 [a]_j \text{ or approximately } \frac{8}{9} [a]_j.$$

The proportion between  $[a]_j$  and  $[a]_D$  varies in different substances owing to their having a different rotatory dispersion.

For sugar solutions  $[a]_j : [a]_D = 1.129$ .

(Very nearly the same as for quartz.)

For camphor solutions in alcohol the ratio is 1.198 and for oil of turpentine 1.243.

**Use of the Polarimeter.**—Figure 27 shows a Schmidt & Haensch polarimeter of the Lippich half-shadow type with divided circle reading by magnification to  $0.01^\circ$ . It is intended for use with a sodium flame, a gas burner for this purpose being supplied with the instrument. This instrument is chosen as being a typical half-shadow instrument and is now in general use. For a complete account of the construction of the many types of polarimeters, the influence exercised by solvents and temperature on the specific rotatory power, Landolt's treatise, "*Das optische Drehungsvermögen*" (F. Vieweg und Sohn, Braunschweig, should be consulted. An English translation has been made by J. H. Long.<sup>1</sup>

In the above illustration  $F$  indicates the telescope,  $ll$  the magnifying glasses,  $nn$  the two verniers,  $K$  the graduated dial,  $A$  the analysing Nicol prism, which is fixed to the revolving graduated dial and to the telescope, and  $P$  the movable polariser, with the graduated segment  $h$  of a circle fixed to it, and  $S$  a small tube for dichromate solution. The sodium lamp is placed at a distance of 36 cm. from the apparatus. It consists of a Bunsen burner (or a Barthel's spirit burner) supplied with a platinum ring on which some pulver-

<sup>1</sup> For a useful, concise description of many types of polarimeter and details of manipulation, Messrs. Baird & Tatlock's catalogue may be consulted with advantage.

ised sodium chloride is placed and made intensely incandescent by means of the non-luminous flame from the burner; the apparatus is pointed towards the brightest part of the yellow flame, which can easily be accomplished by means of the adjuster with the lamp.

The graduated dial, which is made to revolve by means of a knob *T*, is as a rule graduated all the way round. In addition to whole degrees, half and quarter degrees are indicated on the dial; 24 such quarter degrees are divided on the 2 verniers into 25 divisions, therefore a scale mark on the vernier coinciding with any one scale

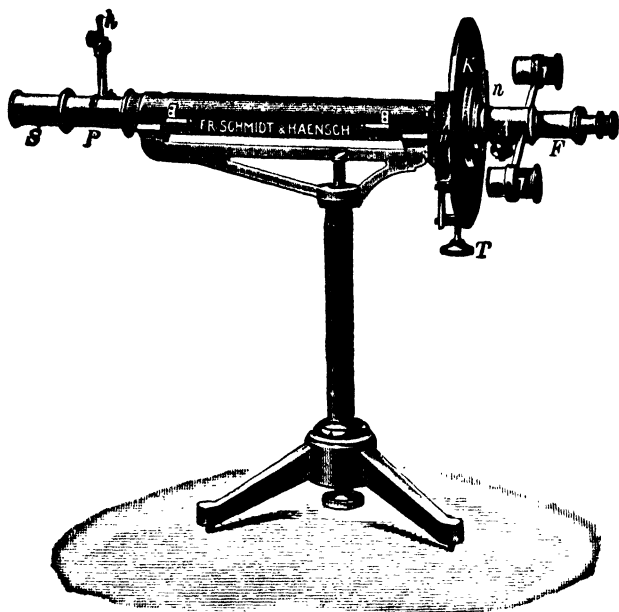


FIG. 27.

mark on the dial indicates  $0.01^\circ$ . Figure 28 shows the inner revolving dial and the exterior vernier; the zero line of the vernier is shown between the  $13.50$  and the  $13.75^\circ$  line of the dial; the  $0.16$  of the vernier, coincides with a line on the dial, therefore the total reading is  $13.50 + 0.16 = 13.66^\circ$ .

If desired, a second scale can be provided on the dial to show directly percentage of some other sugar. This is done by dividing the dial into whole percentages; nine such are divided on the vernier into 10 divisions, so that the vernier reads to  $0.1$  per cent. The

reading is made in the same manner as described above; the beet-sugar scale is based on the standard weight of 26.048 grm. The 100 line (100%) corresponds with a solution of 26.048 grm. of chemically pure sugar in a 100 c.c. flask, examined with the 200 mm. tube. The so-called grape-sugar scale is now rarely used, but if a tube of a certain length (according to the most recent researches 189.4 mm.) is used in connection with the degree scale, the percentages can also be found directly, one degree in this case corresponding exactly to 1% of so-called "grape sugar."

*The adjustment.*—When the above apparatus is well illuminated by the sodium flame, the zero position (the starting-point of all experiments) must first be found; this is indicated by the two halves of the field appearing equally illuminated (equal half-shadows). For this purpose the telescope *F* is focussed on the Lippich's polariser, so that the field presents a perfectly clear, round circle divided into two equal parts by a sharply defined vertical line. If the graduated dial is turned through 3 or 4 degrees to either the right or the left of the zero line, it will be seen that one-half of the field will become lighter, the other half darker.

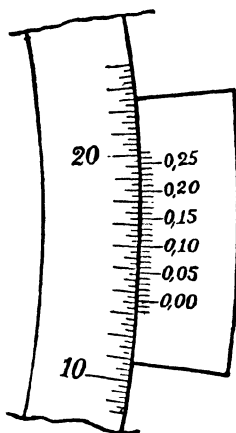


FIG. 28.

The zero position is first adjusted so that the zero line of the circle coincides with the zero line of the vernier. The half-shadow can now be made lighter or darker, according as the polariser is turned to right or left of the zero line by means of the pointer *h*. When the pointer *h* is at zero and the analyser *A* also at zero, both halves of the field appear black. The nearer the pointer is to the zero line the darker the half-shadow and the more sensitive the apparatus. In cases when the solution is not quite transparent, the pointer must be moved slightly away from the zero line so that the field is clear. The instrument is usually so adjusted that the position of the pointer is at  $7\frac{1}{2}^\circ$  when the disc and vernier read exactly  $0^\circ$ . When the pointer is moved the zero of the apparatus changes and no longer corresponds with the zero of the dial. The simplest method is then to take into account the difference in the position of the zero line of the dial and the zero line of the apparatus; or the graduated dial is

moved to  $0^{\circ}$  and the apparatus placed in the zero position by turning the analysing Nicol by the screw *A* to the right or left until the half-shadows are equal in tint.

The following points must be especially observed during a measurement:

1. When the circle has been turned too far and the sensitive range of the apparatus has been lost it is easy to mistake the zero position owing to the light appearing nearly of the same intensity on both sides of the vertical line. Even if the circle is then turned through  $10$  or  $15^{\circ}$  hardly any change will be observed. It is, therefore important, especially when the sample under examination has been placed within the apparatus, to make sure that the transition from light to shade, and *vice versa*, is instantaneous when the circle is turned a few degrees on either side of the zero.

2. When the sample to be tested is inserted, the telescope must first of all be adjusted accurately so that the field is quite clear and equally divided by the vertical line; the circle is then turned until the shades are exactly of the same intensity in the two portions of the field.

*Precautions to be Observed.*—Before the polarimeter tube is filled it should be thoroughly dried by pushing a plug of filter-paper or cotton wool through it, or it should be rinsed several times with the solution to be used. The cover-glasses must be free from scratches and thoroughly clean and dry. Unnecessary warming by the heat of the hand during filling should be avoided; the tube is closed at one end by the screw-cap and cover-glass and grasped at the other with the thumb and finger. The tube is filled with the solution until the meniscus projects slightly above the opening, the air bubbles are allowed time to rise and the cover-glass then pushed horizontally over the end of the tube in such a way that the excess of liquid is carried over the side, leaving the cover-glass exactly closing the tube without air bubbles beneath it and without any liquid on its upper surface. After the cover-glass is in position the tube is closed by screwing on the cap, care being taken that too great a pressure is not exerted, for this might produce a rotatory power in the glass itself and thus give rise to erroneous readings. The rubber washers must therefore be placed in a proper position and the caps screwed in lightly.

Before taking the actual reading, observations are made of the

zero and with a standard quartz plate of known rotation. The mean of several readings is taken and corrected for any deviation of the zero.

In the polarisation of the quartz plates and colourless solutions, difficulty may be experienced in obtaining a complete correspondence of both halves of the field. This may be overcome and the neutral point found, but when it cannot, the ordinary eye-piece of the instrument may be replaced by another which is supplied with the polariscope, and which carries a section of a crystal of potassium dichromate. This removes the difficulty and renders it possible to obtain a field of exact neutrality.

In the latest types of polarimeter the optical field is divided into three parts instead of two as in the half-shadow instruments. Such instruments are more accurate, the equality of the field being capable of a more delicate adjustment.

The arrangement is that shown in Fig. 29. In the zero position 1, 2 and 3 are equally

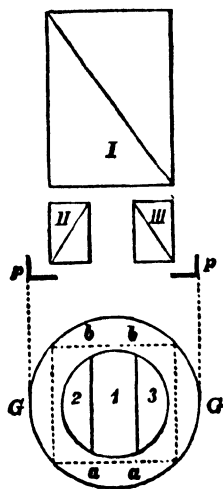


FIG. 29.



FIG. 30.

illuminated while in any other position 1 is dark, while 2 and 3 are illuminated or 1 is bright and 2 and 3 equally dark.

*Ring-shadow* polarimeters (Fig. 30) are a modification of the half-shadow instruments.

They are used in precisely the same way as the ordinary half-shadow polarimeters, but the field of view is divided into two concentric portions instead of into two semicircular segments. With this arrangement the instrument is capable of finer adjustment and the eye is much less fatigued than when using the half-shadow polariser.

Figure 31 shows an improved form (German patent) of tube used for holding the solution to be examined in the polarimeter. Air bubbles enclosed in the tube disappear into the enlargement *a* at one end of the tube and so cease to give trouble during the observation. The caps are of brass and are screwed on as shown. This

pattern of tube is particularly useful in dealing with volatile liquids, such as chloroform.

A specially cheap form of half-shadow polarimeter of the Mitscherlich type is shown in Fig. 32, which reads to  $0.1^\circ$  and has been designed for the estimation of sugar and albumin in urine.

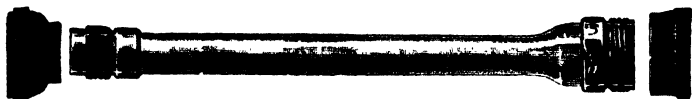


FIG. 31.

Behind the analyser is a small telescope, and behind the polariser a semicircular plate of quartz. The telescope is focussed on to this plate, and the field of vision appears as a circle divided into two halves. A pointer is attached to the analyser, which moves to the

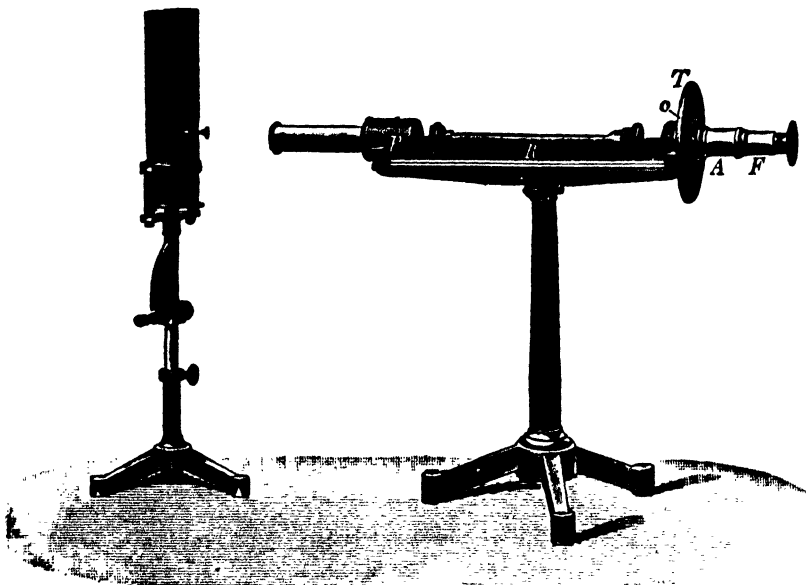


FIG. 32.

right or left on a metal disc divided into angular degrees. A vernier upon which 10 divisions correspond to 9 divisions of the disc enables the observer to read tenths of an angular degree and estimate twentieths.

The instrument is constructed for monochromatic light. A sodium lamp must therefore be used as the source of illumination. The zero point, as in other half-shadow instruments, is found when both halves of the field are of the same tint.

The tube filled with the liquid to be examined is placed in the instrument, and after having focussed the plate by means of the telescope, the pointer is turned to the right or left according to whether the solution is dextro- or lævorotatory, until both halves of the disc are again equally tinted.

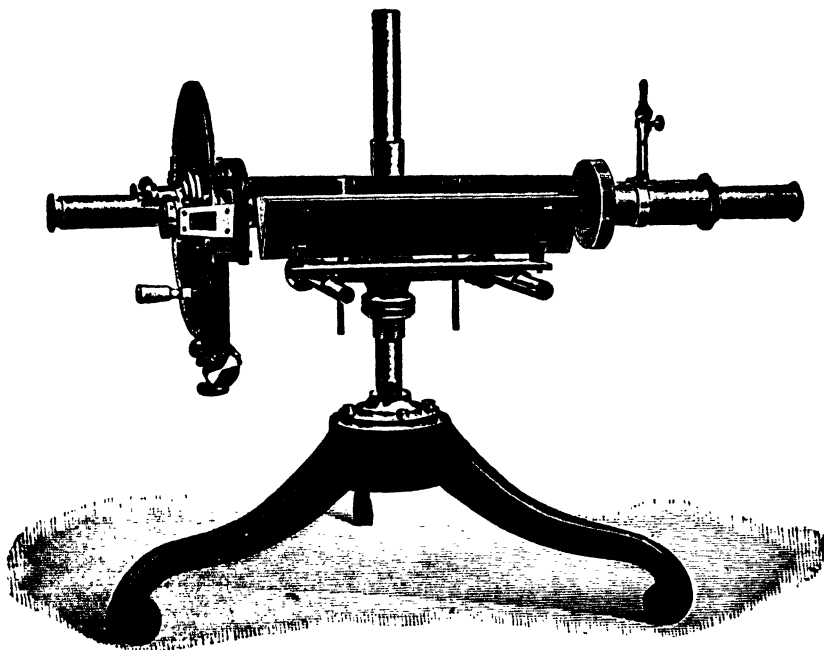


FIG. 33

If the instrument is to be used for general work, a tube of the length of 200 mm. is supplied, and another of 100 mm. for dark coloured solutions, but when used exclusively for urine it is more convenient to have one of 189.4 mm. and another of half that length. These tubes give at once the percentage by volume of sugar and albumin, each degree being equal to 1 grm. in 100 c.c. Albumin polarises to the left to the same extent as glucose does to the right.

The estimation of sugar and albumin in urine is effected in the fol-

lowing manner: the urine, if necessary, is filtered. Should it be too dark coloured to be read in the long tube, the short one is tried, and if still too dark, some extracted animal charcoal is added, and the whole well shaken. In the event of this not effecting decolourisation, 100 c.c. of the urine is introduced into a flask graduated to contain 100 and 110 c.c., basic acetate of lead is added to the 110 c.c. mark, the mixture is then shaken and filtered, and the reading multiplied by 0.11 to correct for dilution. The temperature should be  $15^{\circ}$  to  $20^{\circ}$  C. If the urine is free from albumin the reading corresponds to the percentage of sugar. Should it contain albumin, a few drops of acetic acid are added to 100 c.c. and the solution boiled, cooled, filtered, and made up again to volume at  $15^{\circ}$  to  $20^{\circ}$  C.

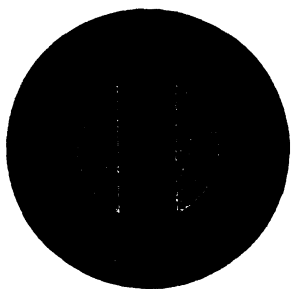


FIG. 34.

As it is necessary to maintain a known constant temperature in order that accurate and comparable measurements can be made with the different types of polarimeters, water or steam jacketed tubes are supplied by the different makers in which the solution to be examined can be heated at an approximately constant temperature. Full information as to these are generally given in the catalogues of dealers in scientific apparatus. The special form of thermostat due to Lowry, which is described in detail elsewhere (page 55), enables a flow of water to be constantly circulated through a polarimeter tube at a temperature which can be maintained constant to within a few thousandths of a degree.

Figure 33 shows a polarimeter made by Hilger, of London, taking tubes 200 mm. in length. The field of view is of the following form (Fig. 34), in which the illumination of the middle strip decreases in intensity when the outer increases, and *vice versa*. The brightness of the illumination can be varied by rotation of the polariser; an index and clamp are provided for the setting of this adjustment.

The table showing the apparent specific rotatory powers of different organic substances which was included in previous editions of this work has been omitted in the present edition, because the value of the specific rotatory power of a substance differs widely with the solvent used and with the temperature and concentration of the solution; numbers expressing the rotatory power are, therefore, misleading

unless the exact conditions observed in measurement are specified. For details concerning all questions of polarimetry and a discussion of the influence exerted by solvents, etc., Landolt's (*Optische Drehungsvermögen*) should be consulted. All available numerical data for the rotatory power of organic substances are included in that work.

**Comparison of Scales of Various Instruments.**—Polarimeters are now usually provided with a scale reading to 100 when a certain quantity of sucrose, called the normal weight, is dissolved in water and made up to 100 c.c. This scale is known as "Ventzke," "Schmidt and Hænsch," and "sugar" scale.

The following factors may be employed for the conversion of data obtained by different instruments:

1 division Schmidt and Hænsch (Ventzke)	0.3468° angular rotation D.
1° angular rotation D	2.8835 divisions Schmidt and Hænsch.
1° angular rotation D	0.7551 division Wild.
1 division Laurent	0.2167° angular rotation D.
1° angular rotation D	4.6154 divisions Laurent.

## ARRANGEMENTS FOR MAINTAINING A KNOWN CONSTANT TEMPERATURE

Several types of thermostat have been devised for the purpose of making physico-chemical measurements in a bath at a known temperature; these are described in treatises on physical chemistry (*e. g.*, Ostwald-Luther, *Physiko-chemische Messungen*; Findlay, *Practical Physical Chemistry*). Lowry (*Trans.*, 1905, 87, 1030 to 1034) gives an account of a series of tests of gas regulators of different patterns used to control the temperature of a bath containing 30 litres of water and well stirred by a paddle driven by a water motor. Two forms of regulator are described, by means of which a known temperature, *e. g.*, 20°, can be maintained in the bath within a few thousandths of a degree over a long period. In a later paper (Lowry, *Trans. Faraday Society*, 1907, 3) a thermostat is described, by means of which a flow of water can be obtained, suitable for heating a polarimeter tube or refractometer prism at a constant known temperature; even when the rate of flow of the water circulation is 4 litres a minute, the temperature in the bath does not vary by more than a few thousandths of a degree. This form of thermostat is particularly serviceable in the examination of *Explosives* (see Vol. III).

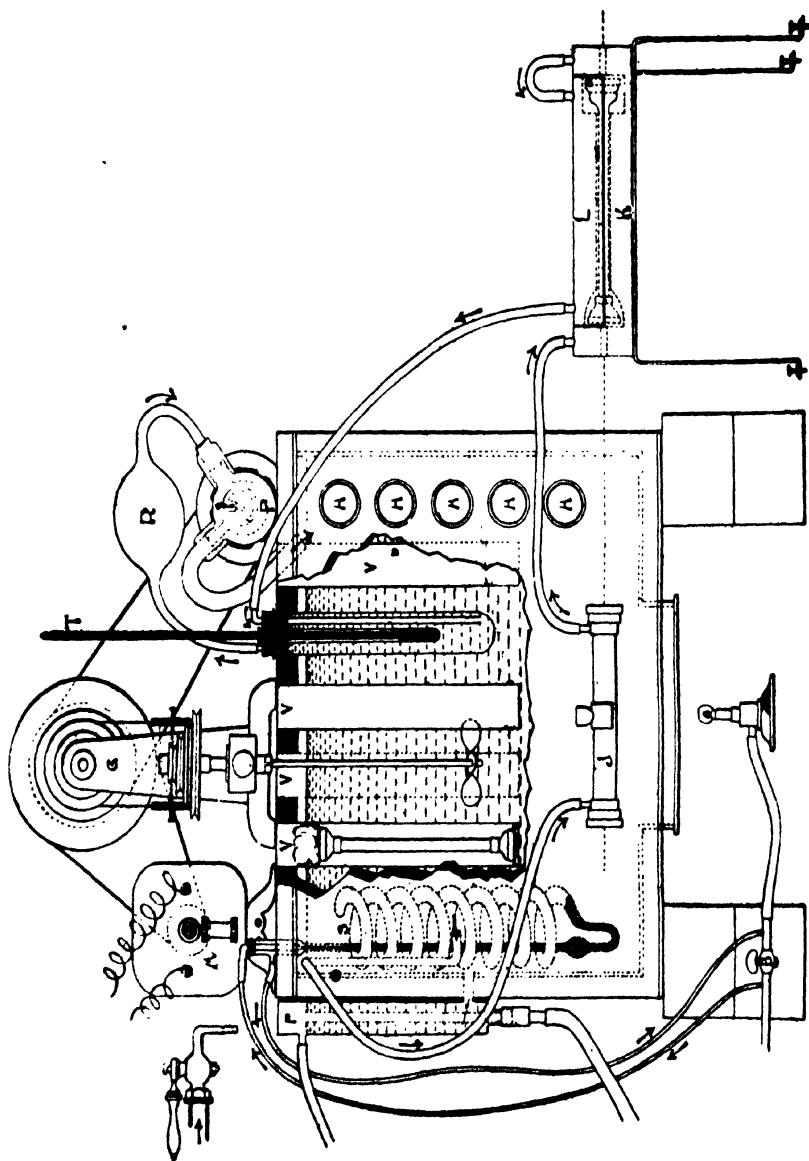


FIG. 35.

This apparatus (made by Messrs. Baird & Tatlock), which the writer has seen in constant use during long periods and which needs practically no attention, is constructed as follows.

The container, as shown in Fig. 35, consists of a large zinc-lined box,  $20 \times 18 \times 16$  in., with a capacity of over 70 litres. The liquid is stirred by a propeller driven by an electric motor. The bulk of well-stirred water and the heat insulation of the wooden box and cover render the regulation of the bath temperature exceptionally easy, with the result that when the gas flame is controlled by a 4-in. spiral (shown at 3 in the figure, and in detail in Fig. 36) the variations are so small that they escape detection even with a thermometer graduated in hundredths of a degree. The heating is effected by means of a small bat's-wing burner placed beneath a copper plate which forms the bottom of the central well of the water-bath; the supply of gas to the burner is controlled on the one hand by the by-pass tap *B*, on the other hand by the spiral *S*.

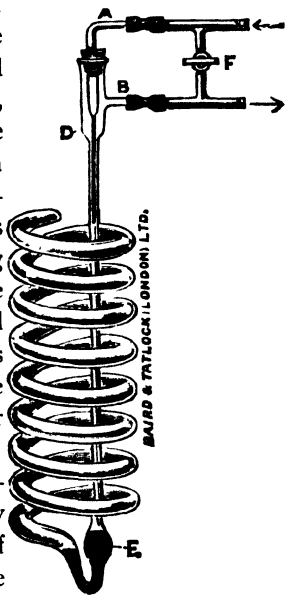


FIG. 36.

The bath is provided with an adjustable overflow *F*; in cold weather it is only necessary to allow an occasional drop of water to drip into the side tube of the bath to maintain the level, but in summer the flow is diverted into the bath itself and greatly increased; ideal conditions are reached when the air temperature is up to  $20^{\circ}$  and the water temperature a few degrees below.

The water circulation is maintained by means of a rotary pump *P* ("Albany" pump) which *sucks* the water out of the bath from a point near to the centre of the regulator, draws it through the various jackets and returns it to the bath. In the figure there are shown (1) an ordinary Schmidt & Haensch jacketed polarimeter tube *J*; (2) a copper water jacket *K* with a removable lid *L*, both supplied with circulating water, designed to take either of the stock patterns of unjacketed polarimeter tubes; when not under observation these can be stored in the bath itself in the vertical tubes *V* or, better, in

the horizontal tubes *H*; the pump may then be stopped, but a slight temperature gradient ( $0.1^{\circ}$  to  $0.2^{\circ}$ ) will appear in the bath if the stirring is also discontinued. The temperature of the return flow can be read by means of a standard thermometer *T*, graduated in hundredths, which dips into a tube of mercury round which the circulating water rapidly passes on its way back to the pump. The rubber bulb *R* serves to take up a part of the thrust of the pump; it usually becomes flattened, but continues to pulsate, when the flow exceeds 1 litre per minute.

The pump is driven from an electric motor *M* through the gearing *G* which carries the propeller and is provided with several adjustments. The speed of the motor is controlled by a lamp-resistance not shown in the figure. When this resistance is short-circuited the pump gives a maximum flow of 4 litres per minute. Under normal conditions a 180-volt, 16-candle-lamp resistance on a 200-volt circuit gives a flow of about 1 litre per minute. The temperature gradient when the bath is at  $20^{\circ}$  and the room at  $15^{\circ}$  is about  $0.01^{\circ}$  per jacket.

When the apparatus described above is used for heating polarimeter tubes or refractometer prisms, the temperature gradient in the leads and jackets is reduced to a minimum by the rapid flow of water which can be increased to any desired extent by speeding up the pump or increasing its size.

A simple form of apparatus made by the Zeiss company for keeping refractometer apparatus at a constant temperature is described under *Refractometers* (p. 31).

A simple thermostat by means of which a sp. gr. or measuring flask can be maintained at a definite temperature, *e. g.*,  $15^{\circ}$ ,  $17.5^{\circ}$  or  $20^{\circ}$ , is constructed by taking a large enamelled iron cylindrical saucepan of a diameter of about 15 ins.; the water contained in this is kept at a constant temperature by means of a 4 in. diameter spiral toluene thermo-regulator, such as is shown in Fig. 36. The water in the thermostat is kept stirred by a small paddle, run by a small water or electric motor.

## ULTIMATE ANALYSIS

When organic substances are heated to redness in the air or in the presence of oxygen-yielding substances, they are generally completely oxidised, the carbon being burnt to carbon dioxide and the

hydrogen to water. Nitrogen is evolved for the most part in the free state, but in some cases partly in combination with oxygen.

The following general outlines may be of service in enabling a suitable method of analysis to be chosen.

**Carbon and hydrogen** are estimated by igniting the substance with dry copper oxide with or without the assistance of a stream of oxygen. The resultant water is absorbed by calcium chloride and the carbon dioxide by potassium hydroxide or soda-lime. In presence of sulphur, chlorine, bromine, iodine or light metals, lead chromate is substituted for the copper oxide. Mercury is liable to distil over into the water-absorption apparatus. In presence of nitrogen the anterior part of the tube is filled with metallic copper. Silver may be substituted for the copper, and has the advantage that it retains halogens, but a high temperature should be employed. The Wetzell potash bulbs (Ber., 1903, 36, 161) are strongly recommended by the writer for the absorption of carbon dioxide (see Fig. 37), owing to their remarkable efficiency. The Hill type of calcium chloride tube (*Proc. Chem. Soc.*, 1906, 22, 87) is also very convenient.

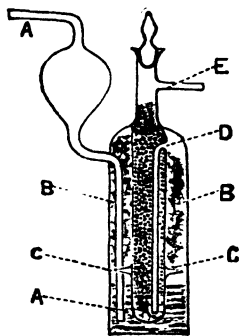


FIG. 37.

Dennstedt (*Anleitung zur Vereinfachung elementär Analyse für wissenschaftliche und technische Zwecke*, 1903; see the series of papers in the *Berichte*, 1897, 30, 1590 and 2861; *Zeit. anal. Chem.*, 1902, 41, 525; 1903, 42, 417; *Zeit. angew. Chem.*, 1905, 18, 1134) has devised a method of combustion in which the substance is volatilised in oxygen and the combustion effected by the aid of platinised quartz or, in the later types of apparatus, of special platinum combusters. The great advantage of the apparatus is that comparatively little heating is required to burn the substance completely and the use of a furnace with a large number of burners is avoided. The method has been still further simplified by J. Walker and T. Blackadder (*Proc. Royal Soc. Edinb.*, 1907-8, 28, 708).

**Nitrogen** may be detected by heating the substance (if a liquid, absorbed by asbestos or sand) with metallic sodium in a narrow test-tube. Cyanide is formed, and may be dissolved out with cold

water. The filtered liquid should be treated with a drop each of ferrous-sulphate and ferric-chloride solutions, and then acidified with hydrochloric acid, when a deep green colouration or Prussian-blue precipitate will indicate that a cyanide was formed.

Most organic compounds give off the whole of their nitrogen in the form of ammonia on ignition with soda-lime. If rich in nitrogen, an addition of sugar should be made to the soda-lime, on each side of the substance to be analysed, so as to expel the air as completely as possible.

Some nitrogenised substances, such as indigo, yield volatile organic bases, instead of ammonia, on ignition with soda-lime. These all resemble ammonia in the fact that their hydrochlorides form double salts with platinum chloride, which on ignition leave 194.8 parts of platinum for 28 of nitrogen.

**Nitro-substitution compounds**, such as picric acid, do not evolve the whole of their contained nitrogen in the form of ammonia when ignited with soda-lime. Addition of sugar improves the result.

**Cyanogen compounds** may be analysed by ignition with soda-lime if a high temperature be ultimately employed. The use of sugar is desirable.

**A general process** for the determination of nitrogen in organic bodies consists in combustion with oxide of copper, passing the gaseous products over red-hot metallic copper or silver, absorption of the carbon dioxide and water by solution of alkali, and measurement of the residual gaseous nitrogen. For details, see (for example) Gattermann's *Praxis des Organischen Chemikers* (7th Ed., Leipzig). (*Practical Methods of Organic Chemistry*, Macmillan.) V. Meyer found that in the case of nitrogenous bodies containing much sulphur it is necessary to replace the oxide of copper by a thick layer of lead chromate, and to conduct the combustion very slowly. The nitrogen obtained should be tested for carbon monoxide. In the case of compounds containing methoxyl and ethoxyl groups the nitrogen evolved may contain large quantities of methane (see Haas, *Proc. Chem. Soc.*, 1906, 22, 81; *Trans.*, 1906, 89, 570).

**Kjeldahl Method.**—For routine work in organic analysis, the Kjeldahl method is now generally used. The original method employed special oxidising agents, but in most cases Gunning's modification is used. The reagents and procedure in the standard (A.O.A.C.) Kjeldahl-Gunning method are as follows:

*Potassium Sulphate*.—A coarsely powdered form free from nitrates and chlorides should be selected.

*Sulphuric Acid*.—This should have a sp. gr. 1.84 and be free from nitrogen compounds.

*Standard Acid*.—N/2 sulphuric or hydrochloric acid, the strength of which has been accurately determined.

*Standard Alkali*.—N/10 ammonium hydroxide, sodium hydroxide, or barium hydroxide, the strength of which in relation to the standard acid must be accurately determined.

*Strong Sodium Hydroxide Solution*.—Five hundred grm. should be added to 500 c.c. of water, the mixture allowed to stand until the undissolved matter settles, the clear liquor decanted and kept in a stoppered bottle. It will be an advantage to determine approximately the quantity of this solution required to neutralise 20 c.c. of the strong sulphuric acid.

*Indicator*.—Cochineal solution is recommended by the A.O.A.C., but methyl-orange, azolitmin, methyl red and sodium alizarinmono-sulphonate are satisfactory. Phenolphthalein is unsuitable for titration of ammonium compounds.

*Combined Digestion and Distillation Flasks*.—Jena-glass round-bottomed flasks with a bulb 12.5 cm. long and 9 cm. in diameter, the neck cylindrical, 15 cm. long and 3 cm. in diameter, flared slightly at the mouth.

*Process*.—From 0.7 to 3.5 grm., according to the proportion of nitrogen, are placed in a digestion flask. Then 10 grm. of powdered potassium sulphate and 15 to 25 c.c. (ordinarily about 20 c.c.) of the strong sulphuric acid are added and the digestion conducted as follows: the flask is placed in an inclined position and heated below the b. p. of the acid during from 5 to 15 minutes, or until frothing has ceased. Excessive frothing may be prevented by the addition of a small piece of paraffin. The heat is raised until the acid boils briskly. A small, short-stemmed funnel may be placed in the mouth of the flask to restrict the circulation of air. No further attention is required until the liquid has become clear and colourless or not deeper than a pale straw-colour.

When Kjeldahl operations are carried out in limited number, the arrangement shown in Fig. 38 is satisfactory. A double-Y, terracotta drain-pipe, about 20 cm. internal diameter, is connected by an elbow directly with the chimney-stack. The digestion flasks are

supported as shown in a rough sketch (not drawn exactly to scale). Two flasks can be operated at once. The central opening is convenient for other operations producing fumes. Openings not in use are closed by circles of heavy asbestos.

The apparatus shown in Fig. 39 is used when many determinations are made. As corrosive vapours are given off, it must be placed

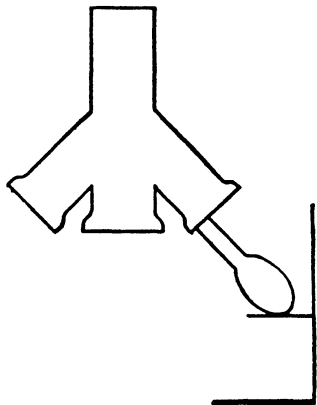


FIG. 38.

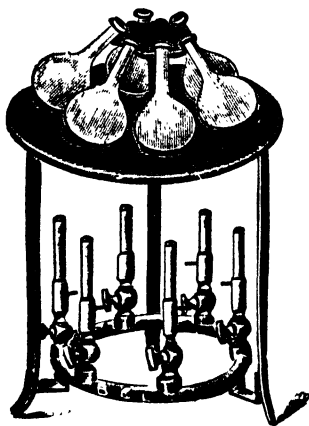


FIG. 39.

under a hood. The central opening in the ventilating pipe shown in Fig. 38 will be satisfactory; the mouths of the flasks should be well inside the margin of the pipe.

When the liquid has become colourless or very light straw-yellow, it is allowed to cool, and diluted by the cautious addition of 200 c.c. of water. Granulated zinc, pumice-stone, or 0.5 gm. of zinc dust is added. 50 c.c. of the strong sodium hydroxide solution, or sufficient to make the reaction strongly alkaline, should be slowly poured down the side of the flask so as not to mix at once with the acid solution. It is convenient to add to the acid liquid a few drops of the indicator solution, to show when the liquid is alkaline, but it must be noted that strong alkaline solutions destroy some indicators. The flask is attached to the condensing apparatus and is shaken so as to mix the alkaline and acid liquids. The receiving flask should have been previously charged with a carefully measured volume of the  $N/2$  acid (10 c.c. diluted with water to 100 c.c. is a convenient amount). A few drops of the indicator solution should also be added. The distillation is conducted until about 150 c.c.

have passed over. The acid is then titrated with standard alkali, and the amount neutralised by the distilled ammonia determined by subtraction. Each c.c. of  $N/2$  acid neutralised is equivalent to 0.007 nitrogen.

The distillation in this operation requires care, as the amount of ammonia formed is determined by its neutralising power, hence solution by the alkali of the glass will introduce error. Common glass is not satisfactory. Block-tin is the best material for the Kjeldahl-Gunning form, but Moerrs has shown that it is not adapted to the methods in which mercury oxide is employed. He found that Jena-glass tubes resist the action of the alkali. The distillates should be titrated promptly; on standing for some hours some alkali may be taken up from the flask.

The most satisfactory condensing arrangement for general laboratory use is a copper tank of good size, through which several condensing tubes pass. Such an arrangement as applied to Kjeldahl distillations is shown in Fig. 40, which is a rough sketch, not drawn to scale. The flask is the standard

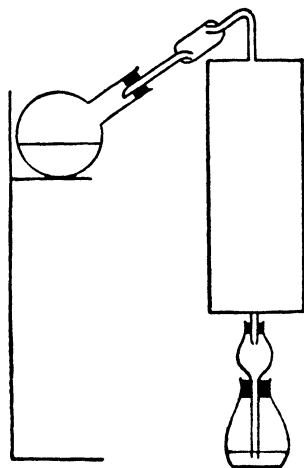


FIG. 40.

Jena-glass distilling flask, about 9 cm. diameter; the tank should be high enough to allow of a condensing tube 60 cm. long. The connection of this with the receiving flask is made by means of a bulb tube to allow for occasional drawing back of the liquid. The cork through which this tube passes into the flask must not fit closely, as opportunity must be given for expansion of the air. The safety-tube connecting the distilling flask with the condenser should terminate a little below the water level in the tank. The apparatus may be satisfactorily heated by a low-temperature burner. To avoid spurting of the boiling liquid, it is usual to interpose a safety-tube between the distilling flask and the condenser. Many forms have been suggested. That shown in Fig. 41 is most in use.

In some analyses (as in the case of pepper) the Kjeldahl-Gunning

method must be replaced by Arnold's modification: One grm. of the sample is mixed with 1 grm. of crystallised copper sulphate and 1 grm. of mercuric oxide. The potassium sulphate-sulphuric acid mixture as given above is added and the mass heated cautiously until frothing ceases, when the temperature is raised and the digestion completed.

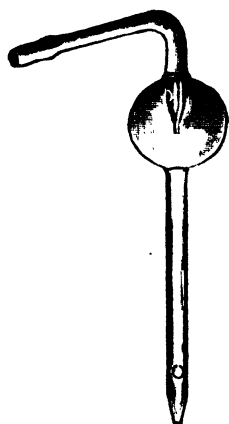


FIG. 41.

The liquid is diluted for distillation, 50 c.c. of a solution of commercial potassium sulphide (40 grm. to 1000 c.c.) are added, and sufficient sodium hydroxide as usual. The liquid is liable to bump.

**Modification for Nitrates.**—If nitrates are present in the material, the weighed sample is well mixed with 35 c.c. of sulphuric acid containing 2%, by weight, of salicylic acid, and the mass shaken frequently during 10 minutes; 5 grm. of sodium thiosulphate are added and 10 grms. of potassium sulphate. The mixture is heated very gently until frothing ceases and then according to the usual method. The nitrogen in the distillate will include that derived from the nitrogen of the nitrates.

**Chlorine, bromine and iodine** are detected in an organic substance by heating it with metallic sodium in a small glass tube, dropping the tube, whilst hot, into distilled water, filtering the solution so obtained from the fragments of glass and testing with silver nitrate after acidifying with pure nitric acid. If nitrogen is present in the compound silver cyanide is formed in the above process; in such cases it is best to heat a little of the substance with pure lime in place of the sodium in the above test and, after dissolving the product in pure dilute nitric acid, to test for the halogen in the solution. The halogens are best estimated by Carius' method which consists in completely oxidising the substance by heating it with fuming nitric acid (of sp. gr. 1.5) in a sealed tube containing about 1.5 times the theoretical quantity of silver nitrate. The method is described in most text-books of practical organic chemistry (*e. g.*, Gattermann's *Practical Methods of Organic Chemistry*) and is very accurate. In most cases the decomposition of the compound is complete after heating 6 hours at 200–205° C.; but it is safest to heat the tube, after releasing the

pressure, during another 6 hours at 250–300°, so as to insure complete decomposition.

Plimpton and Groves determine the halogens in volatile organic substances by burning the substance gradually in a bunsen flame, placed under a trumpet-shaped tube, and absorbing the products of combustion in solution of sodium hydroxide which is subsequently acidified with nitric acid and precipitated by silver nitrate. The test analyses by this method are highly satisfactory, and the process is rapid and simple.

**Sulphur, phosphorus and arsenic** may be detected by igniting the substance with pure soda-lime mixed with an oxidising agent, such as potassium chlorate, mercuric oxide, or sodium peroxide. The residue is tested for sulphates, phosphates and arsenates. The process may be made quantitative. Another method is to heat the substance in a sealed tube with nitric acid of 1.5 sp. gr. The sulphur, phosphorus and arsenic are converted, respectively, into sulphuric, phosphoric and arsenic acids. Sulphur is also easily recognised in the solution obtained by heating the substance with sodium in testing for a halogen; it is only necessary to put a drop of the solution on a silver coin when, if sulphur is present, the coin is blackened owing to the formation of silver sulphide.

Sulphur, phosphorus and arsenic are readily estimated by oxidising the substance with nitric acid, as in Carius' method, this treatment giving rise to sulphuric acid, phosphoric acid and arsenic acid, respectively (see page 64).

**Metals** usually remain in the residue obtained on igniting the organic substance in the air. Metals of the alkalis and alkaline earths are usually left as carbonates, but when sulphonic groups are present sulphates are formed; phosphates or haloids may be formed when phosphorus or halogens are present. Heavy metals are usually left as oxides, except silver, gold and platinum, which will remain in the free state. Arsenic, antimony and other metals, when existing in volatile compounds, may be completely volatilised.<sup>1</sup>

**Mercury** will usually be wholly volatilised. It may be estimated in all instances by igniting the substance with soda-lime, and collecting and weighing the mercury which distils over.<sup>2</sup>

<sup>1</sup> Perhaps a more satisfactory method of procedure is to heat the organic substances with concentrated sulphuric acid as in the Kjeldahl-Gunning method of estimating nitrogen. Mercury may be detected or estimated in the

**Oxygen** may be detected by ignition of the substance in a stream of hydrogen, when water will be formed. By igniting the substance in a stream of chlorine, or in admixture with potassium chloroplatinate, carbon dioxide will be formed if oxygen be present. Hydrochloric acid and chlorine may be respectively absorbed by solutions of lead nitrate and stannous chloride, and the carbon dioxide passed into baryta water or potassium-hydroxide solution. In the great majority of cases the oxygen of organic bodies is estimated "by difference."

### MOISTURE, CRUDE FIBRE AND ASH

These are data of *proximate analysis*, frequently required in connection with commercial organic analysis.

**Moisture** may be either hygroscopic or molecular. The former is always influenced by atmospheric conditions, the latter not usually. In most cases in commercial organic analysis, the hygroscopic moisture is alone important. In ordinary cases the operation is conducted in a water- or air-oven at atmospheric pressure, but vacuum drying ovens are now much used, and for some analyses an atmosphere of hydrogen is necessary. Soxhlet's oven, in which a solution of common salt in water is used, permits of the employment of a temperature slightly higher than  $100^{\circ}$ .

Moisture is usually estimated with sufficient accuracy, provided other volatile bodies are not present, by heating the material (solids should be finely divided) in a flat dish on the water-bath or in the water-oven until it ceases to lose weight. Flat platinum dishes from 4 to 8 cm. in diameter and 0.5 cm. high are well adapted to this work. They should rest on porcelain or asbestos rings. Nickel dishes are often applicable, especially the broad shallow crucible covers made in dish form. Dishes of glass—especially the shallow (petri) dishes used for microbe culture—and porcelain are suitable; aluminum and tin less so. The drying of a liquid will be facilitated by using an absorbent material, such as pure quartz sand, powdered asbestos or pumice-stone. These materials should be extracted with dilute hydrochloric acid, well washed and well dried before use. The

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acid solution. Arsenic and antimony in organic combinations are looked for similarly. Excess sulphuric acid may be neutralized.—*Amer. Ed.*

quantity used should be rapidly weighed, preferably in the dish in which the operation is to be carried out. It is advisable to cover the dish with a nearly flat, thin watch-glass in all the weighings. By a few trials a glass can be selected which fits fairly close to the rim of the dish and restricts evaporation or absorption of water. It is often convenient to weigh a small stirring-rod with the dish and absorbent.

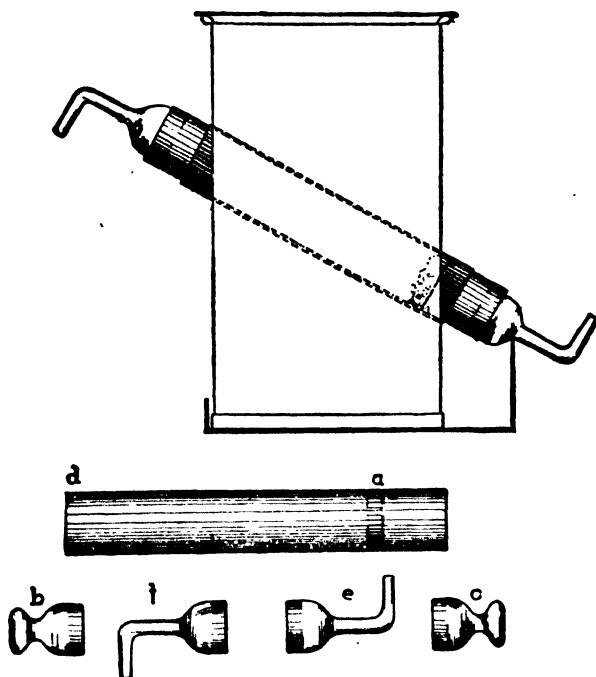


FIG. 42.

In many cases a liquid can be measured directly into the dish, the residue being recorded in grm. per 100 c.c. or other suitable ratio.

Syrupy and gelatinous liquids or those containing much solid matter, especially if this be somewhat difficult to dry, may often be more satisfactorily treated by diluting a weighed portion with several times its weight of water, evaporating a measured or weighed amount of the dilute liquid, and calculating the amount of residue in the original substance.

The ordinary water-bath and water-oven need no description.

The temperature of materials heated on the former is usually much less than  $100^{\circ}$ ; in the latter, slightly below  $100^{\circ}$ .

The following are some convenient special forms of drying oven:

Figure 42 shows a drying oven for use with a current of hydrogen. The apparatus was designed by Caldwell for estimating moisture, ether-extract, and crude fibre as prescribed by the A.O.A.C., the three data being determined on the same sample.

The bath is made of copper and is 24 cm. long, 15 high, and 8.5 broad. It stands in a piece of sheet-copper bent at right angles along the sides, as shown in the end view; on one side this vertical part need not be over 1 cm. high, just enough to project a little up the side of the bath, which rests snugly against it; along the other side it projects upwards, at a little distance from the side of the bath, about 15 mm., and to about the height of 4 cm.; opposite each of the tubes of the bath a slot is cut in this vertical part, which serves then as a shoulder against which the glass tube rests when in place, to keep it from slipping down and out of position.

The tube for containing the substance has at the zone *a* three small projections on the inner surface, which support a perforated platinum disc of rather heavy platinum foil carrying the asbestos filter. This tube is 13 cm. long and 23 mm. inner diameter, and weighs, with its closed stoppers, about 30 grm.

The filter is readily made in the same manner as the gooch filter, the tube being first fitted to a suction flask by an enlargement of one of the holes of the rubber cork or, better still, by slipping a short piece of rubber tube over it, of such thickness that it will fit tightly in the mouth of a suction flask provided with a lateral tube for connection with the suction. A thin layer of asbestos is sufficient; if it is too thick, the gas and ether will not flow through readily.

About 2 grm. of the substance are put in this tube, previously weighed with the stoppers *b* and *c*, and the weight of the substance accurately determined by weighing tube and contents. The stoppers are removed, a band of thin asbestos paper is wound around the end *d* of the tube, a little behind the slight shoulder at the rim, as many times as may be necessary to make a snug fit, when this tube is slid down into the copper tube in the bath, thus preventing circulation of air between the glass and the copper tubes that would retard the heating of the former; the stopper *e* is put in the lower end of the tube for connection with the hydrogen supply, and the stopper

*f* in the upper end; this latter stopper is connected by rubber tube with a glass tube slipping easily through one of the holes of a rubber cork closing a small flask, containing a little sulphuric acid, into which this tube just dips; when as many tubes as are to be charged are thus arranged in place and the hydrogen is turned on, the even flow of the current through the whole number is secured by raising or lowering a very little the several tubes through which the outflow passes, so as to get a little more back pressure for one, or a little less for another, as may be found necessary. When the drying is supposed to be completed, the tubes are weighed again with their closed stoppers, and so on.

For ether-extraction the unstoppered tube with contents is put directly into the extractor.

Carr and Osborne have made an extended series of investigations as to the estimation of water, and find that more accurate results may be obtained if the operation be conducted under a diminished pressure at a temperature not exceeding  $70^{\circ}$ . Under these conditions it was found possible to dehydrate l  vulose completely without decomposition. The oven is made of a section of metal tubing, from 15 to 20 cm. in diameter and 30 to 40 cm. long. One end is closed air-tight by a brass end-piece, brazed or attached by a screw. The other end is detachable and is made air-tight by ground surfaces and a soft washer. On the top are apertures for the insertion of a vacuum-gauge and for attachment to a vacuum-apparatus, thermostat and thermometer. The aperture for admission of air or hydrogen is best placed at the fixed end. The oven may be heated by a single burner, but a series of small jets is preferable. The metal should be protected by sheet asbestos. The temperature of the oven can be kept uniform by a gas regulator or by attention to the lamp (see page 72).

The method of operating is as follows: Clean pumice-stone of two grades of fineness is used, one that just passes through a 1 mm. mesh and one that passes through a 6 mm. mesh. These are digested with hot 2% sulphuric acid, washed by decantation until the wash-water is free from acid, placed, wet, in a sand crucible and heated to redness. When the water is expelled, the material may either be placed hot into a desiccator or directly into the drying dishes. In loading the dishes, place a thin layer of dust over the bottom of the dish to prevent the material to be dried from coming in contact

with the metal; over this layer place the larger particles, nearly filling the dish. If the stone has been well washed, no harm can result from placing the dish and stone over the flame for a moment before transferring to the desiccator preparatory to weighing.

If the material to be dried is a thick liquid, it is diluted until the sp. gr. is in the neighbourhood of 1.08 by dissolving a weighed quantity in a weighed quantity of water. (Alcohol may be substituted in the case of material not precipitable thereby.) Of this, 2 to 3 grm. may be distributed over the stone in a dish the area of which is in the neighbourhood of 20 sq. cm., or 1 grm. for each 7 sq. cm. of area. The material is distributed uniformly over the pumice by means of a pipette weighing-bottle (weighing direct upon pumice will not answer), ascertaining the weight taken by difference.

The dishes are placed in the oven, which should be maintained at a pressure of not more than 125 mm. of mercury. The temperature must not exceed about 70°. All weighings must be taken with the dish covered by a close-fitting plate. The open dish must not be exposed to the air longer than absolutely necessary. Weighings may be made at intervals of from 2 to 3 hours.

In the laboratory of the United States Geological Survey a sheet-iron or nickel basin about 10 cm. in diameter and 3 cm. deep is set upon an iron plate which is heated directly by the burner. A platinum or pipe-clay triangle rests in the basin and supports the dish containing the liquid to be evaporated. It is stated that almost any liquid can be evaporated in this way without sputtering. The temperature, however, is liable to be too high for many organic substances.

Parsons has obtained good results in the drying of sensitive organic substances by the following method: A perfectly neutral petroleum oil, free from animal or vegetable oils and mineral substances, sp. gr. 0.920, flash test 224°, fire test 260°, b. p. about 288°, is heated to about 120° for some time and preserved in a well-stoppered vessel. A quantity of oil about 6 times that of the weight of the substance to be dried is heated in an evaporating dish in a drying oven to a temperature of 115°, and then weighed. The weighed portion of the substance is put into the oil; if it be very moist, it is added in small portions. Slight effervescence will usually occur, and the mass should be kept in the drying oven for a short time after effervescence has ceased. The evaporating dish containing the oil and

substance is weighed; the loss is moisture. The whole operation may be completed in less than half an hour.<sup>1</sup>

#### CONSTANT TEMPERATURE OVENS

An approximately constant temperature can be maintained in an ordinary hot-air oven heated by gas, by controlling the supply of gas by means of a suitable thermo-regulator, *e. g.*, a Reichert mercury regulator of the type shown in Fig. 43.

A vapour bath such as that devised by Victor Meyer and shown in Fig. 44, is often convenient for drying a substance at a known constant temperature; with such a bath almost any definite temperature can be maintained by choosing a suitable liquid to be vapourised in the outer jacket. For example, chloroform, petroleum, benzene, toluene, xylene, aniline, naphthalene gives a series of definite temperatures on a rising scale. The same principle is applied in the Abati drying oven (Vereinigte Fabriken für Laboratoriums-bedarf) shown in Fig. 45.

A suitable solvent is kept vigorously boiling in the flask below the oven by means of the bunsen burner. With this apparatus it is very easy to change from one constant temperature to another by simply changing the flask and solvent.

Several types of ovens heated by electricity are also in use.

<sup>1</sup>The most satisfactory method of determining water in most classes of organic substances is by distillation with water saturated xylene, according to the xylol method or by the use of a fraction of petroleum distillate, generally known as petroleum spirit, which is intermediate in gravity and boiling range between kerosene and gasoline. It is in fact a heavy naphtha. Connect the flask containing the 10 gm. substance in which water is to be estimated and 75 c.c. of the water saturated xylol or petroleum spirit with a condensor, which must be perfectly dry. Heat the flask gradually in a bath of cylinder oil and distil the xylol and water slowly until the xylol comes over clear, collecting the distillate in a funnel tube with a stem graduated to 0.1 c.c. The bulk of the water comes over with the first 10 c.c. of distillate. After the distillation is completed, wash down the condenser with water-saturated xylol and tap the funnel tube gently until any small drops of water clinging to the sides are brought down to the bottom. Read the volume of water. Each 0.1 c.c. is equivalent to 1 %  $H_2O$  in the sample using a 10-grm. sample. This latter method was first suggested by the *Amer. Editor* (see *J. Ind. & Eng. Chem.*, II, 1910, 66).—*Amer. Ed.*

## VACUUM DRYING OVENS

In organic analysis it is often convenient to dry a substance in a vacuum at a lower temperature than the b. p. of the solvent impregnating the substance.<sup>1</sup> A convenient form of apparatus for drying in a vacuum (Siderski, *Zeit. anal. Chem.*, 1890, 280) is shown in Fig.

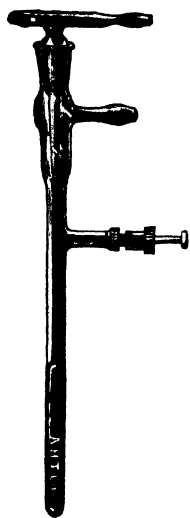


FIG. 43.

47; it is made by the V.F.f.L. An arrangement made by the same firm for evaporating in a vacuum is shown in Fig. 46.

**Crude Fibre.**—The proximate constituents included in this term are principally forms of cellulose, but the method given herewith, which is that of the A.O.A.C. yields a residue containing notable amounts of other substances:

2 grm. of the substance, well extracted with ether (see under "Extraction"), are mixed in a 500 c.c. flask with 200 c.c. of boiling water containing 1.25% of sulphuric acid; the flask is connected with an inverted

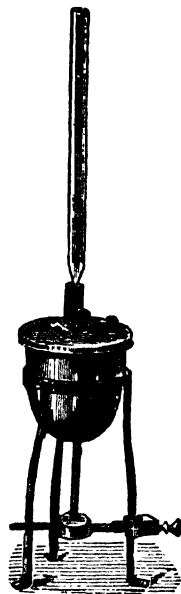


FIG. 44.

condenser, the tube of which passes only a short distance below the rubber stopper of the flask. The liquid is brought to the b. p. as rapidly as possible and maintained there for 30 minutes. A blast of air conducted into the flask may serve to reduce the frothing of the liquid. The mass is filtered, washed thoroughly with boiling water until the washings are no longer acid; the undissolved substance rinsed back into the same flask with the aid of 200 c.c. of boiling water containing 1.25% of sodium hydroxide nearly free from sodium carbonate; again brought to the b. p. rapidly and maintained there for 30 minutes as directed above.

The liquid is filtered by means of a gooch crucible; washed with boiling water until the washings are neutral to phenolphthalein; dried at 110°; weighed and then incinerated completely and again weighed. The loss of weight is crude fibre.

<sup>1</sup> The Freas Vacuum ovens are largely used in the United States and Canada. —*Amer. Ed.*

The filters used for the first filtration may be linen, glass, wool, asbestos, or any form that secures clear and reasonably rapid filtration. Hardened-paper filters may serve. The sulphuric acid and sodium hydroxide must be made up of the specified strength, deter-

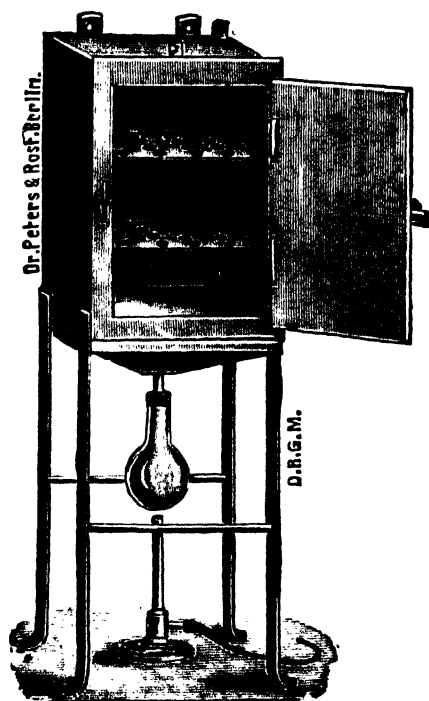


FIG. 45.

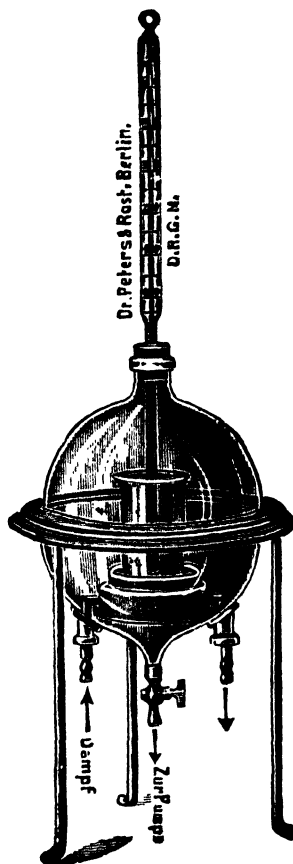


FIG. 46.

mined by titration. The material must be ground very fine and the preliminary extraction with ether must not be omitted. It is probable that carbon tetrachloride could be advantageously substituted for ether in the preliminary extraction.

Crude fibre should not be called cellulose.

**Ash.**—The method of detecting and estimating the mineral constituents of an organic substance usually consists simply in igniting a known weight of the body in free contact with the air, and weighing the residue or *ash*.

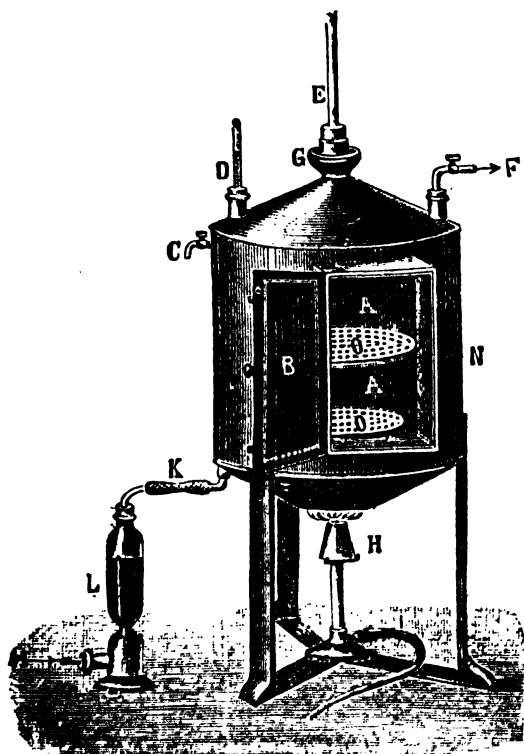


FIG. 47.

The most satisfactory method of estimating the *ash* of organic substances is to conduct the ignition in a platinum tray or flat capsule placed in a gas-muffle maintained at the lowest temperature compatible with combustion. The tray should be supported on a row of pieces of tobacco-pipe stem or other non-conducting substance, so as to avoid over-heating from contact with the bottom of the muffle. If a bunsen burner is employed to effect combustion, similar care should be taken to avoid over-heating. If too high a temperature is employed there is great danger of loss by volatilisation of chlorides

or carbonates and additional trouble may arise from fusion of the remaining ash, with consequent enclosure of particles of unburnt carbon. By keeping the temperature as low as possible, and avoiding local heating, nearly all organic substances can be burnt completely and without difficulty. In obstinate cases, the unconsumed matter may be mixed with ammonium nitrate or moistened with a strong solution of the salt and then reignited. Addition of pure mercuric oxide is also useful occasionally, or the refractory matter may be mixed with a known weight of dry ferric oxide and again ignited.

In very many instances the difficulty of effecting complete combustion and the danger of loss by volatilisation may be wholly overcome by moistening the substance to be ignited, or the carbonaceous residue therefrom, with strong sulphuric acid. This converts the readily fusible and volatile chlorides and carbonates into the more fixed *sulphates* of the alkali metals, and on ignition complete combustion will readily ensue. This method is used in ascertaining the percentage of metal in sulphonates and some other salts. It is necessary to moisten the ash with a drop of sulphuric acid and reignite, so as to get rid of any sulphides left after the first ignition. For obtaining the ash of animal matters, it is desirable to treat the substance in a porcelain crucible with a mixture of strong nitric and sulphuric acids. This dissolves and destroys the organic matters before ignition, and, on evaporating the liquid to dryness and igniting the residue, complete combustion ensues, and a white "sulphated ash" is readily obtained. The same modification of the usual method of determining the ash of plants may be pursued with advantage in many cases, the starch and cellulose being first converted into oxalic acid, which the sulphuric acid decomposes into carbon oxides and water, so that after evaporation of the acid there is but little organic matter left to ignite.

Sulphuric acid is almost always employed in determining the ash of commercial sugars, a deduction being made from the weight obtained for the increase due to "sulphation."

Besides being in excess of the true ash, the "sulphated ash" will contain no chlorides or carbonates. Phosphoric and silicic acids are not affected by the treatment.

The estimation of ash may be facilitated by igniting the charred residue in a current of oxygen. The complete combustion of the carbon is frequently prevented by the formation of a glaze of fused

mineral matter. In many cases this difficulty may be avoided by allowing the charred mass to cool, washing it with distilled water and collecting the washing through a small, nearly ashless filter; the washed residue is then burned white, the watery solution added, and evaporated to dryness.

It must be remembered, however, that carbonate in the ash is usually the skeleton of the salts of organic acids present in the original substance. Many analysts deduct the carbon dioxide in the ash from the total weight obtained, and report the difference as "true ash." A similar correction is often made for the "sand and carbon" left on treating the ash with dilute acid, the sand being merely an accidental impurity and not a true constituent of the plant, and the carbon being simply due to incomplete combustion of the organic matter.

*The ordinary constituents* of the ash of natural organic substances are potassium, sodium, calcium, magnesium, manganese and iron which exist as oxides, carbonates, sulphates, phosphates, silicates and chlorides. Traces of other elements exist normally in certain cases, but the foregoing are those to which attention is generally directed. In algæ notable traces of bromides and iodides occur, while some other cryptogams contain aluminum. Common flowering plants and animal tissues used for human food do not contain appreciable amounts of aluminum, but clay being a common ingredient of soils, aluminum compounds may be present as adventitious material. Copper is widely distributed, occurring in minute amount in wheat flour and some of the viscera (*e. g.*, liver) of domestic animals. It is a constant ingredient of the circulating fluid of the lobster. Lately, barium has been found as a notable ingredient in the ash of some plants from the cattle feeding districts of the western United States. It has also been found in Egyptian wheat. Zinc is present in a few rare cases.

*Analysis of Ash.*—Ash analysis may be effected by the ordinary methods of mineral analysis, but it should be borne in mind that the ash of wheat and other cereals is apt to contain pyrophosphates and these must be converted into orthophosphates by fusing the ash with alkaline carbonate before the ordinary process for phosphoric acid can be employed. Titration of chlorine by silver nitrate solution (with potassium chromate as indicator) cannot be effected with accuracy,

unless the phosphates have been previously removed by precipitating the aqueous solution of the ash with calcium nitrate.

In many cases it is of service to ascertain the proportion of the total ash which is soluble in water. This is most conveniently done by igniting and weighing the insoluble matter and deducting the weight found from that of the total ash previously determined. The aqueous solution can then be used for the estimation of the chlorides, alkalinity and other data. Some analysts apply the term "soluble ash" to the ash left on igniting the residue obtained by evaporating to dryness the filtered aqueous solution of the substance. This is not identical with the soluble portion of the ash of the whole substance, and should be called in preference the "ash of the aqueous extract."

The *alkalinity*, or capacity of the ash for neutralising acid, is a useful indication. It is commonly expressed in terms of  $K_2O$ , and is estimated by titrating the filtered aqueous solution of the ash with standard acid.

**Poisonous metals** are apt to occur as impurities in certain commercial organic products, being accidentally introduced during the process of preparation. The objectionable metals most commonly occurring are arsenic, lead, copper, zinc and tin, and in ordinary cases the search may be limited to these.

**Liquids.**—In some cases, as, for instance, vinegar and lemonade, the metallic impurities may be sought for in the original liquid, but in others it is desirable to evaporate the liquid carefully to dryness, ignite the residue, and test the resultant ash. The evaporation should be conducted in porcelain. 100 c.c. of such liquids as beer, cider or vinegar will usually suffice for the examination, but sometimes the use of considerably larger volumes is desirable. Towards the end of the evaporation, an addition of strong nitric and sulphuric acids should be made, the quantity used depending on the amount of organic matter to be destroyed. The evaporation is then carefully completed and the residue ignited at a low red heat. After cooling, the ash is moistened with nitric acid and 1 drop of sulphuric acid, and again ignited. It is then again treated with a few drops of nitric acid, which is evaporated off cautiously, the process being stopped directly acid fumes cease to be copiously evolved. The residue is then treated with hot water, and the

solution filtered, when the following scheme of analysis should be followed.<sup>1</sup>

<b>Aqueous Solution</b> may contain copper, zinc, iron. Add excess of ammonia and filter		<b>Residue</b> may contain lead, tin. Wash, and pour boiling solution of ammonium acetate on the filter	
<b>Precipitate</b> may contain iron, phosphates.	<b>Filtrate</b> , if blue, contains copper. Divide into two portions.		<b>Residue.</b> Ignite filter paper, fuse ash in porcelain crucible with potassium cyanide, dissolve product in water, filter, boil insoluble residue with strong hydrochloric acid; dilute, and treat clear solution with mercuric chloride. A white silky precipitate of mercurous chloride is due to tin.
	1. Acidify with acetic acid and add potassium ferrocyanide. Brownish precipitate or colouration is indicative of copper.	2. Heat to boiling, and add potassium ferrocyanide. White precipitate or turbidity indicates zinc.	
		<b>Solution</b> Acidify with acetic acid, and add potassium chromate. A chrome yellow precipitate indicates lead.	

Minute traces of copper are perhaps best detected by introducing a steel knitting needle into the slightly acidified and tolerably concentrated aqueous solution of the ash, removing it after some hours, cautiously rinsing it in water, and then immersing it in dilute ammonia, with free contact of air. The copper precipitated on the iron will pass into solution, and may be detected by acidifying the ammoniacal liquid with acetic acid and adding potassium ferrocyanide, when a purple or brownish colouration will be produced if a trace of copper be present.

**Solids** may be examined for traces of the foregoing poisonous metals in precisely the same way as liquids which have been concentrated to a small bulk by evaporation.

The detection of zinc and copper in food articles has become of considerable importance lately, in view of the use of colouring matters containing these substances, and the tendency to restrictive legislation concerning such use. Much attention, for example, has been given to the detection of zinc in dried apples, in consequence of the efforts of the German government to prohibit the importation of American dried apples, under the allegation that they were dangerously contaminated with zinc derived from the plates on which the drying is conducted. Wiley, in a bulletin published by the United States Department of Agriculture, has given the results of an investigation into this question; in some cases he obtained results

<sup>1</sup> Volatile metals should be tested after treatment as suggested in footnote on p. 65.—*Amer. Ed.*

differing materially from those obtained upon the same samples by the German chemists.

In most cases, especially in examining food and household articles, an amount of arsenic sufficient to be of sanitary significance may be detected by Reinsch's test, using a liberal allowance of hydrochloric acid, since the more high oxidised forms of arsenic (arsenates) do not give the reaction in the presence of small amounts of hydrochloric acid. Reinsch's test cannot be applied in the presence of active oxidising agents, such as chromates, chlorates or nitrates. Processes for examination of beer, glucose and foods for arsenic will be described in the sections devoted to such substances.

The examination for arsenic and poisonous metals in cases of suspected poisoning does not come within the scope of this work and will not be described.

The detection of alum and other mineral adulterants of flour and bread is described under "cereals."

## BEHAVIOUR OF ORGANIC SUBSTANCES WITH SOLVENTS

In the proximate analysis of plants and other complex substances of organic origin, a systematic treatment with solvents is a most valuable means of separating different classes of compounds from each other. The systematic use of solvents has been worked out very thoroughly by Dragendorff and others, whose methods will be described in greater detail in future sections. In proximate organic analysis only a limited use is made of the stronger acids so largely employed in mineral analysis, while the use of alcohol, ether, chloroform and other organic solvents is greatly extended.

**Exhaustion of Organised Tissues by Solvents.**—In assaying commercial organic substances it is often requisite to effect as perfect an exhaustion as possible of an organised tissue of some active principle or valuable constituent existent therein. This is the case in the assay of cinchona barks for alkaloid, of seeds and oil-cakes for oil, and of sugar-cane and beet-root for sugar. In such cases the cells which contain the principles to be extracted are only incompletely ruptured by the most careful pounding or crushing of the sample, and hence solvents can only act on the contents through the cell walls, and the resultant solution can only pass through the cell walls

by diffusion. This often renders the process of exhausting organised tissues very tedious, while the difficulty is enhanced by the fact that economy and convenience of subsequent treatment often render it desirable or necessary to use a very limited quantity of solvent. Under these circumstances, an apparatus which will act almost automatically and allow of complete exhaustion by a small quantity of solvent possesses great advantages.

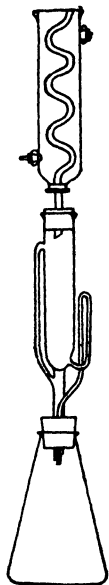


FIG. 48.

**Soxhlet's Tube.**—For the automatic exhaustion of a substance by a volatile solvent, no better arrangement has been described than an ingenious device of Szombathy, commonly called Soxhlet's apparatus (Fig. 48). The substance to be extracted is inclosed in a plaited filter or cylinder of filter-paper, or if it be coarse it is sufficient simply to place it loose in a large test-tube, having an aperture at the bottom closed by a plug of glass-wool. Thus arranged, the tube or filter with its contents is placed in a Soxhlet tube, having a little glass-wool at the bottom, and adapted by means of a cork to a flask containing the solvent. A vertical condenser is adapted to the upper end of the Soxhlet's tube, and the solvent kept boiling by a suitable source of heat. In the case of petroleum spirit, ether or other volatile and inflammable solvent, this should be a water-bath kept hot by a small flame or an electric stove. As the solvent boils it is condensed and falls on the substance to be extracted, remaining in contact with it until both the inner and outer tubes are filled to the level of the syphon, when

the solution passes off into the flask, to be redistilled and recondensed, and so on until the process is judged to be complete.<sup>1</sup> With a proper arrangement of the source of heat, the extraction goes on regularly and automatically. On changing the flask and replacing the inner tube by one containing a fresh sample, the apparatus is ready to be used for another extraction.

The paper thimbles made by Schleicher and Schüll are convenient for use with extraction apparatus.

<sup>1</sup>The completion of this process is sometimes very tedious. Cocoa powder requires about 24 hours for complete extraction. Where there are no cell walls for the solvent to penetrate a few hours are sufficient.—*Amer. Ed.*

A very simple and convenient form of exhaustor, adapted either for extraction or repercolation, has been described by Dunstan and Short (*Pharm. Jour.*, 1882-3, [3], 13, 664). It consists of two glass tubes, the wider of which is drawn out at one end. The narrower and somewhat shorter tube fits into the outer one with much margin, and is also drawn out in such a way as to allow the end to protrude from the drawn-out end of the wider tube when the smaller is inserted therein. At the point where the outer tube commences to contract it is indented on opposite sides, by which means two ledges are formed within the tube which serve as supports for the narrower tube.<sup>1</sup> The inner tube serves to contain the substance to be exhausted. The lower drawn-out end of the wider tube is fitted by a cork to the flask containing the volatile solvent, while the upper end is connected with a condensing arrangement.

J. West-Knights has described a form of exhaustor which may be conveniently used when the quantity of material to be extracted is somewhat small (*Analyst*, 1883, 8, 65). A percolator is made by cutting off the bottom from a test-tube of suitable size, and blowing a hole in the side of the tube about an inch from the top. A disc of filter-paper or fine cambric is tied over the lower end of the tube. The substance to be extracted is placed in the tube, and kept in its place by some glass-wool and a perforated disc of metal, and the tube with its contents then fixed by a cork to the lower end of the tube of a vertical condenser. This is adapted by a larger cork to the neck of an ordinary flask containing the volatile solvent, on heating which the vapour passes through the hole in the side of the test-tube up into the tube of the condenser, where it is liquefied. The condensed liquid drops right into the test-tube, percolates through the substance to be extracted, and falls to the bottom of the flask, to be again volatilised. As the percolator is inside the flask, its contents are kept constantly at the b. p. of the solvent, and, the action being continuous and automatic, very rapid exhaustion may be effected.

The "flow" extractor devised by Burgess and shown in the sketches in Figs. 49 to 51 is more effective than the Soxhlet for many purposes, the substance being kept covered with the solvent in use and a flow of clean solvent continuously passing through it. The joints are ground-in joints.

<sup>1</sup> The indentations are made by gently pressing each side of the tube when red-hot with a pair of crucible tongs.

Other forms of exhauster have been contrived by Church, Drechsel, Angell, Thoms, Thresh (*Pharm. Jour.*, 1884-5, [3], 15, 281) and others, but those described will be found sufficient for most purposes.

**Employment of Immiscible Solvents.**—In mineral analysis this method finds but few applications, but in proximate organic analysis one of the most valuable means of effecting separations con-

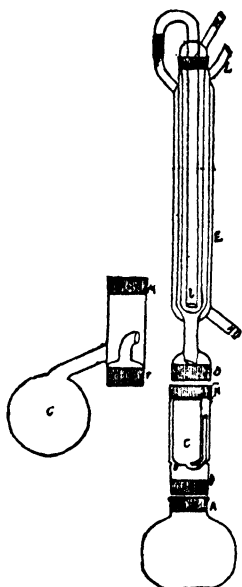


FIG. 49.

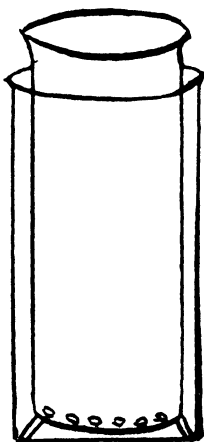


FIG. 50.

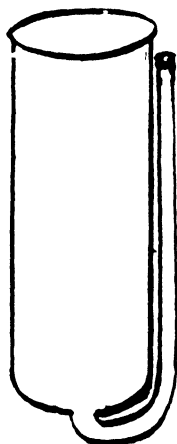


FIG. 51.

sists in agitating the solution of a substance in one solvent, with another solvent insoluble or only slightly soluble in the former liquid. Under these circumstances, the dissolved body is distributed between the two solvents in proportions which are dependent on the relative solubility of the substance in the two media, and the relative quantities of the two media employed. Thus, it may be supposed that, if a substance be 99 times more soluble in chloroform than in water, and its aqueous solution be shaken with an equal measure of chloroform, 99% of the whole substance will pass into the chloroform. On separating this layer, and again agitating the residual aqueous liquid with an equal quantity of chloroform, 99% of the remaining substance will be dissolved, thus making the exhaustion practically complete. The distribution of a solid between two non-miscible

solvents is dealt with in works on Physical Chemistry, for example, Nernst's "*Theoretische Chemie*."

In making a proximate analysis by means of immiscible solvents, much of the success in practice depends on the care and skill with which the manipulation is conducted. The most convenient apparatus for effecting the treatment consists of a pear-shaped (Fig. 52) or cylindrical glass separator, furnished with a tap below and a stopper at the top. The tube below the tap should be ground obliquely so as to prevent loss of liquid by imperfect delivery. Supposing that it be desired to effect the separation of a substance from an aqueous liquid by agitation with ether, the former is introduced into the separator, of which it should not occupy more than one-third, acid or alkali added as may be desired, and next a volume of ether about equal to that of the aqueous liquid. The stopper is then inserted and the whole thoroughly shaken together for a minute or two, and then set aside. As a rule, the contents will readily separate into two well-defined layers, the lower of which is aqueous and the upper ethereal. Sometimes separation into layers does not occur readily, the liquid remaining apparently homogeneous, forming an emulsion or assuming a gelatinous consistency. In such cases separation may sometimes be induced by thoroughly cooling the contents of the separator. In the case of ether, the separation may always be effected by adding an additional quantity of ether and reagitating, or, when the employment of a sufficient excess of ether is inconvenient or impracticable, the addition of a few drops of alcohol, followed by a gentle rotatory motion of the liquid, will almost invariably cause separation to occur promptly.

Separation having taken place, the aqueous layer should be run off by the tap into another separator, where it can again be agitated with ether to insure the complete removal of the substance to be dissolved therein. The ethereal liquid remaining in the first separator should be shaken with a fresh quantity of alkaline or acidified water, which is then tapped off as before, and the remaining traces removed by treating the ether with a little pure water. This having in turn been run off to the last drop, the ethereal solution can next be

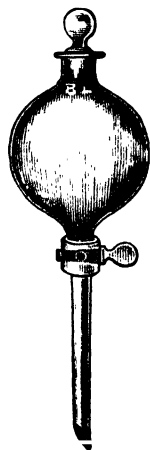


FIG. 52.

removed by the tap, but a preferable plan is to pour it out of the top of the separator, by which means any contamination by the traces of water adhering to the sides of the glass will be avoided.

When amyl alcohol, benzene, or petroleum ether is employed, the manipulation is the same as that just described; but when chloroform is used or a mixture containing a considerable proportion of that solvent, the aqueous liquid forms the upper stratum, and the chloroform solution can at once be removed by the tap.

The tendency to form an obstinate emulsion is greater when the aqueous liquid is alkaline, and is often very troublesome when chloroform, benzene, or petroleum spirit is substituted for ether. In such cases the employment of a larger quantity of the solvent sometimes causes separation, but, when admissible, a better plan is the addition of ether. This answers very successfully for the isolation of strychnine, which is nearly insoluble in unmixed ether, but readily soluble in a mixture of equal measures of ether and chloroform. This solvent is heavier than water and is capable of very extensive application.

It is evident that the treatment can be repeated any number of times requisite to ensure the complete extraction of substances having a limited solubility in the solvents employed, and these can themselves be varied in a systematic manner, as is done in Dragendorff's method for the separation of alkaloids and other active principles.

The separation of immiscible solvents is in many cases promoted by rapid rotation. The centrifugal machines employed for the rapid analysis of milk do not usually give sufficient speed for this purpose, but some of the smaller forms intended for clinical work can be operated at very high velocity, and by their use a small amount of such mixtures can often be separated rapidly and thoroughly.

Care must be taken to ascertain the purity of the solvents used in these methods, especially when toxicological investigations are being conducted. Vaughan has reported a case in which a sample of ether made by a prominent house contained a poisonous substance in such amount that the residue left by the evaporation of 50 c.c. of the ether killed a guinea-pig in a short time. Chloroform often contains carbonyl chloride, in which case it is also acid owing to the presence of hydrogen chloride.

Figure 53 shows a special apparatus for use with solvents lighter than water.

The cylinder *A* should hold about 1000 c.c. Two openings are not necessary, since both tubes may pass through the cork, but the arrangement shown is more convenient. 600 c.c. of the solution are placed in the cylinder, 300 c.c. of solvent added and the mixtures well shaken. The rest of the apparatus is then attached. The flask *B* has a capacity of 200 to 300 c.c.; the solvent in it is heated by a waterbath. The vapour passes by *a* into *b*, the condensed liquid flows to the bottom of *A* and rises through the solution; the upper layer returns through *c* into *B*. The tube *c* should not extend into the liquid in *B*. A small quantity of aqueous liquid may collect at intervals in *B* and should be removed.

The table on page 86 shows the behaviour of the principal organic substances on treatment with water, made slightly acid or alkaline, and solvents immiscible therewith, such as ether, chloroform, amyl alcohol, benzene, and petroleum ether. It must not, however, be supposed that the immiscible solvents can be employed indifferently, as some of the substances are removed from their aqueous solutions by one solvent, but are unaffected by others owing to their limited solubility therein. This is especially the case with the alkaloids and glucosides, and hence the table must merely be regarded as showing the general tendency, their behaviour when treated with the individual solvents being deferred for full description later.

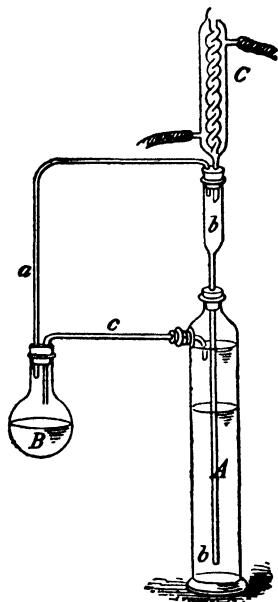


FIG. 53.

TABLE SHOWING THE BEHAVIOUR OF ORGANIC SUBSTANCES WITH IMMISCIBLE SOLVENTS

On agitating the substance with water, acidified with sulphuric acid, and a suitable solvent immiscible therewith (such as ether, chloroform, amyl alcohol, benzene or petroleum spirit), the following distribution will occur:

<p><b>The acidified aqueous liquid</b> will contain <i>carbohydrates, soluble alkaloids and acids, organic bases, proteins</i>, which may be further separated by adding a moderate excess of sodium hydroxide, and again shaking with a suitable immiscible solvent, when there will be obtained:</p>	<p><b>The immiscible layer</b> will contain <i>hydrocarbons, oils, various acids, resins, colouring matters, phenols, glucosides</i>, which may be further separated by agitating the liquid with water containing sodium hydroxide, when there will be obtained:</p>
<p><b>In the alkaline aqueous liquid—</b> <i>Carbohydrates</i>: as sugars, gums, dextrin. <i>Soluble Alcohols</i>: as methyl alcohol, ethyl alcohol, glycerol. <i>Soluble Acids</i>: as acetic, oxalic, lactic, malic, tartaric, phenol-sulphonic. <i>Certain Alkaloids or Organic Bases</i>: as curarine, urea, glycosine, solanine, and possibly cinchonine, morphine, and pyridine. <i>Certain Colouring Matters</i>: as indigo products. <i>Proteins and their Allies</i>: as albumin, casein, gelatin.</p>	<p><b>In the alkaline aqueous liquid—</b> <i>Fatty Acids</i>: as stearic, oleic, valeric. <i>Various other Acids</i>: as benzoic, salicylic, phthalic, meconic. <i>Acid Dyes and Colouring Matters</i>: as picric and chrysophanic acids, alizarine, aurine, bilrubin. <i>Acid Resins</i>: as colophony, phenols: as cartholic and cresylic acids, thymol, creosote. <i>Certain Glucosides</i>: as santonin, cantharidin, picrotoxin.</p> <p><b>In the immiscible layer—</b> <i>Solid Hydrocarbons</i>: as paraffin, naphthalene, anthracene. <i>Liquid Hydrocarbons</i>: as petroleum products, rosin-oil, benzene. <i>Essential Oils</i>: as turpentine. <i>Nitro-compounds</i>: as nitrobenzene. <i>Ethers and their Allies</i>: as ether, chloroform, compound ethers, nitroglycerin. <i>Fixed Oils, Fats, and Waxes</i>. <i>Neutral Resins and Colouring Matters</i>. <i>Camphors</i>: as laurel-camphor, bornol, menthol. <i>Alcohols</i>: insoluble or nearly insoluble in water; as amyl and cetyl alcohols, cholesterol. <i>Certain Glucosides</i>: as saponin, digitain, santonin. <i>Certain Weak Alkaloids</i>: as caffeine, colchicine, narcotine, piperine, theobromine.</p>

# ALCOHOLS.

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REVISED BY L. M. BURGHART

The simple aliphatic alcohols which have acquired industrial importance form the subject matter of this chapter. Methyl and ethyl alcohols are of course by far the most important. As in previous editions acetone is included because of its close relation to methyl alcohol, both in the usual method of its production and also in the methods of its analysis. The development of fusel oil refining in the United States in recent years and the appearance of isopropyl and normal butyl alcohols as commercial products have led to the introduction of a few paragraphs dealing briefly with the higher aliphatic group, including normal and iso-propyl, normal and iso-butyl, and amyl alcohols.

The examination of ethyl alcohol from the industrial, as distinguished from the beverage, point of view has been gradually getting more attention in recent years. Methods of examination of alcohol intended for industrial purposes are, however, very much unsettled. Naturally the tendency is to depend on the more or less established methods which were developed originally in connection with beverage alcohol, but they are not always suitable. These methods are given in detail in the chapter dealing with Potable Spirits. It is intended to give in this section only such supplementary information as may be useful in connection with industrial alcohol.

## METHYL ALCOHOL

*Carbinol—Methanol—Purified Wood Spirit—CH<sub>3</sub>OH.*—The chief source of methyl alcohol is the aqueous portion of the distillate which results from the dry distillation of wood. In crude or partially purified condition the alcohol has a characteristic raw and disagreeable odour due to compounds associated with it. When these are thoroughly removed, the pure alcohol resulting is a colourless, mobile liquid with a pleasant, very faint odour.

From experiments in which stress was laid on constancy of b. p. throughout the distillation Timmermanns (*Bull. soc. belg. chim.*, **24**, 244) found a maximum and minimum b. p. at 760 mm. of 64.71–64.69° with an observed change of 0.30° per 10 mm. Young and Fortey (*J. Chem. Soc.*, 1902, **81**, 717) reported 64.71 and Gyr (*Ber.*, 1908, **41**, 4322) 64.56 at 760. The b. p. may be used as a test for reputed 100% methyl alcohol. The low number given by some workers, who were at great pains to dehydrate the spirit, is due no doubt to acetone which depresses it notably, because of the formation of a constant boiling mixture of 88 parts acetone and 12 parts methyl alcohol which distils at 55.7° at 760 mm. (Lecat, *L'azeotropisme*, 1918, p. 80). The m. p. according to Ladenburg and Krugel (*Ber.*, 1899, **32**, 1821) is –94.9°.

*Sp. Gr. Tables:* The sp. gr. of methyl alcohol according to the recalculation of the Klason and Norlin figures (*Archiv. Chem. Min. Geol.*, 1906, **2**, No. 24, 6) by Doroshevskii and Rozhdestvenski, *J. Russ. Phys. Chem. Soc.*, **41**, 951; *Chem. Centr.*, 1910, **I**, 155; C.A., 1910, **4**, 1404–5) is 0.796472 at 15/15; 0.79578 at 15/4. Other values are: Young (*Sci. Proc. Roy. Dublin Soc.*, **12**, 374–443; C.A., 1911, **5**, 407) 0.81000 at 0/4; Timmermanns (*loc. cit.*) 0.81017 at 0/4; Gyr (*loc. cit.*) 0.79649 at 15/15; U. S. Bureau of Standards Tables (*Standard Density and Volumetric Tables*, Circular No. 19, 1916, p. 22–24) 0.79647 at 15/15; 0.79577 at 15/4. Klason and Norlin recalculated the earlier methyl alcohol tables of Dittmar and Fawsitt (*Trans. Roy. Sci.*, 1889, **33**, ii, 509) and an abridgement of this recalculation was given in the fourth edition. The U. S. Bureau of Standards selected the data of Doroshevskii and Rozhdestvenski for their recalculation, which is given below. The sp. grs. of this table are based on vacuum-corrected weights, hence the same precautions as given under the use of the ethyl alcohol tables apply.

Methyl alcohol is produced in the United States in grades varying from the denaturing wood spirit, containing 18–30% acetone, with considerable amounts of allyl alcohol and other impurities, up to the best grade of acetone-free methyl alcohol, which is also practically water-free. Methanol, Columbian Methanol, Columbian Spirit, and other trade names are commonly used. It finds use as a denaturant of ethyl alcohol—the lower grade as a complete denaturant, and the so-called “commercially pure” methyl alcohol (99%) in some of the special formulæ. It is used also as a solvent for varnish gums, as a

## SP. GR. AT 15°/15° C. OF MIXTURES (BY VOLUME) OF METHYL ALCOHOL AND WATER

Per cent. methyl alcohol by volume at 15° C.	Sp. gr. at D 15°/15° C.	Differences	Per cent. methyl alcohol by volume at 15° C.	Sp. gr. at D 15°/15° C.	Differences
0	1.00000	0.00149	50	0.93326	0.00171
1	0.99851	0.00148	51	0.93155	0.00173
2	0.99703	0.00143	52	0.92982	0.00176
3	0.99560	0.00138	53	0.92806	0.00180
4	0.99422	0.00139	54	0.92626	0.00183
5	0.99283	0.00137	55	0.92443	0.00187
6	0.99146	0.00135	56	0.92256	0.00189
7	0.99011	0.00134	57	0.92067	0.00190
8	0.98877	0.00131	58	0.91877	0.00195
9	0.98746	0.00125	59	0.91682	0.00199
10	0.98621	0.00125	60	0.91483	0.00201
11	0.98496	0.00126	61	0.91282	0.00203
12	0.98370	0.00123	62	0.91079	0.00206
13	0.98247	0.00122	63	0.90873	0.00210
14	0.98125	0.00122	64	0.90663	0.00213
15	0.98003	0.00119	65	0.90450	0.00216
16	0.97884	0.00118	66	0.90234	0.00220
17	0.97766	0.00118	67	0.90014	0.00224
18	0.97648	0.00118	68	0.89790	0.00229
19	0.97530	0.00117	69	0.89561	0.00234
20	0.97413	0.00118	70	0.89327	0.00239
21	0.97295	0.00118	71	0.89088	0.00244
22	0.97177	0.00119	72	0.88844	0.00248
23	0.97058	0.00119	73	0.88596	0.00250
24	0.96939	0.00119	74	0.88346	0.00254
25	0.96820	0.00120	75	0.88092	0.00256
26	0.96700	0.00120	76	0.87836	0.00258
27	0.96580	0.00121	77	0.87578	0.00266
28	0.96459	0.00121	78	0.87312	0.00272
29	0.96338	0.00122	79	0.87040	0.00280
30	0.96216	0.00125	80	0.86760	0.00286
31	0.96091	0.00125	81	0.86474	0.00294
32	0.95966	0.00128	82	0.86180	0.00297
33	0.95838	0.00130	83	0.85883	0.00301
34	0.95708	0.00132	84	0.85582	0.00306
35	0.95576	0.00133	85	0.85276	0.00309
36	0.95443	0.00135	86	0.84967	0.00321
37	0.95308	0.00138	87	0.84646	0.00332
38	0.95170	0.00141	88	0.84314	0.00343
39	0.95029	0.00143	89	0.83971	0.00348
40	0.94886	0.00145	90	0.83623	0.00354
41	0.94741	0.00148	91	0.83269	0.00362
42	0.94593	0.00150	92	0.82907	0.00369
43	0.94443	0.00152	93	0.82538	0.00375
44	0.94291	0.00155	94	0.82163	0.00391
45	0.94136	0.00157	95	0.81772	0.00409
46	0.93979	0.00159	96	0.81363	0.00421
47	0.93820	0.00163	97	0.80942	0.00428
48	0.93657	0.00164	98	0.80514	0.00432
49	0.93493	0.00167	99	0.80082	0.00435
50	0.93326	...	100	0.79647	

raw material in organic synthesis, and in the manufacture of formaldehyde, dimethylaniline, and of various esters, such as methyl acetate. The danger of its appearance as an adulterant in tinctures, etc., and the occasional reckless attempts to use denatured alcohol as a beverage are chiefly responsible for the wealth of methods for its detection.

**Detection of Methyl Alcohol.**—An attempt has been made to select those methods which are not only quick and reliable, especially for the detection of methyl alcohol in the presence of ethyl, but which have also been given sufficiently wide use so that their limitations are generally recognized. As a matter of safety it is important not to depend on any one method. Results by one method should be confirmed by other methods as different in principle as possible, as Gettler (*Jour. Biol. Chem.*, 1920, **61**, 311) has emphasized in his study of a large number of methods. In exacting cases the writer has come to depend on a combination of four methods applied to the same alcohol, or purified distillate, namely, the modified Vorisek, the Riche-Bardy, the Prussic Acid, and either U.S.P. or Chapin's modification of Denigès' method as described below. It is important to check method and manipulation by parallel tests on known mixtures of a composition approximately that of the sample being examined.

Most of the methods for detecting methyl alcohol depend on oxidation to formaldehyde and detection of the latter. A carefully regulated oxidation procedure should be used, rather than oxidation with a heated spiral of copper wire. Under the rough treatment of the latter method other substances than methyl alcohol may produce considerable formaldehyde and make the test uncertain in cases involving less than 5% methyl alcohol.

The following discussion of Denigès' method, given in the words of the IVth Revision (Vol. IX, pp. 1-3) emphasizes the importance of careful regulation of all conditions of the experiment when attempting to detect methyl in the presence of ethyl alcohol.

*Denigès' Method According to Simmonds:* "By this method, due originally to Denigès (*Compt. rend.*, 1910, **150**, 832; C.A., 1910, **4**, 1425) the presence of 0.2% methyl alcohol (or about 4% of completely denatured alcohol, C.D. No. 1) in ethyl alcohol can be detected with certainty within 20 minutes and only twice as much time is required to estimate the proportion of methyl alcohol with sufficient exactness for most purposes. Denigès claims that the method will detect as little as 0.1% of methyl alcohol in ethyl, and Simmonds (*Analyst*, 1912, **37**, 16) has shown that it can be made even more sensitive. The writer's own experience confirms this, but it will be seen from a subsequent paragraph that there is risk in attempting to push the sensitiveness too far, and the method

as now to be described will not detect much less than 0.2% of methyl alcohol expressed as a percentage of the total alcohols. This is sensitive enough for almost any purpose, as the addition of less than 5% of industrial spirit to ethyl alcohol would probably represent too small a saving to the sophisticator to be worth his while. The subsequent notes show the direction in which the test should be modified to increase its sensitiveness, should this be necessary.

"Simmonds (*loc. cit.*) describes the test as follows: "The alcoholic liquid is first purified, where necessary, either by the method of Thorpe and Holmes (p. 102) or by other suitable means. It is then diluted with water until it contains 10% total of alcohol by volume.

"To 5 c.c. of this prepared liquid, contained in a wide test-tube, are added 2.5 c.c. of permanganate solution (2%), and then 0.2 c.c. of strong sulphuric acid. When the reaction has proceeded for 3 minutes, 0.5 c.c. of oxalic solution (9.6 grm. crystals in 100 c.c.) is added. On shaking, the liquid becomes clear and nearly colourless. 1 c.c. of strong sulphuric acid is now run in and well mixed with the solution, which is finally treated with 5 c.c. of Schiff's reagent. A violet colour is developed in the course of a few minutes unless mere traces of methyl alcohol were present, when 20 or 30 minutes may be required.

"A preliminary experiment carried out as described serves to detect the presence of methyl alcohol and to give some idea of the quantity. According to the indications thus obtained, another part of the prepared liquid is further diluted, if necessary, with ethyl alcohol of 10% strength until it contains from 0.001 to 0.004 grm. methyl alcohol in 5 c.c., and the experiment is repeated side by side with two or more standards for comparison. These contain 0.001, 0.002, 0.003 etc., grm. methyl alcohol in 5 c.c. of 10% ethyl alcohol."

"The above description of the test requires so little amplification that the writer has preferred to leave it in the words of Simmonds, who first sought to establish the test here. Simmonds does not give the formula of the Schiff's solution to be used, nor does he, in the writer's view, lay sufficient stress on the necessity of measuring all the reagents used, a point of great importance. Curiously enough, the strength of the oxalic acid, which is of least importance, he states with great precision. He points out, it is true, that the function of the sulphuric acid added immediately before the Schiff's reagent is to suppress any colouration due to acetaldehyde, but does not

say that the intensity of colouration, due to a stated amount of formaldehyde, is closely dependent on the final concentration of acid, as it actually is. Again, the amount of formaldehyde formed from methyl alcohol depends on the exact conditions of oxidation, and even ethyl alcohol itself may yield formaldehyde if the conditions are not strictly controlled. This last fact has settled the fate of many similar methods. In first describing this test Denigès pointed out that the use of too high a concentration of sulphuric acid with the permanganate would give rise to formaldehyde, even with pure ethyl alcohol.

"The formula adopted in the preparation of the Schiff's solution is important, as it affects the final concentration of acid. The first (strongly acid) solution described on page 326 is quite useless for the purpose, as are many less acid ones, *e. g.*, that of Mohler (Fremy's *Encyclopedie chimique*, 1892, X, 11, 278) perhaps that most used in the examination of potable spirits. The other solution, made from magenta base and sulphurous acid, serves well and was used in all the experiments described below.

"A 9.6% solution of oxalic acid, as recommended by Simmonds, deposits crystals at ordinary temperatures, but with a cold saturated solution of oxalic acid, Schiff's solution of the character recommended above, and precise measurement of quantities, Simmonds' directions may be followed.

"How nearly the quantities need to be measured and how they should be varied to increase or diminish the sensitiveness of the test will be evident from the following results obtained in the writer's laboratory.

"The amount of permanganate used must be rigidly adhered to in quantitative work, since it determines the amount of formaldehyde formed and the final intensity of colour. The use of 2 c.c. in place of 2.5 c.c. reduces the final colour about 30%, whilst the use of 5 c.c. more than doubles the sensitiveness of the test, when all other conditions are kept the same, except that the use of 5 c.c. of permanganate necessitates the subsequent use of about 1 c.c. instead of 0.5 c.c. of oxalic acid solution. Provided all the other standard conditions are rigidly adhered to, this is the simplest and safest way of increasing the sensitiveness of the test, as ethyl alcohol under these conditions yields no formaldehyde and no colour, but the permissible latitude in all the other measurements necessarily becomes narrower.

"The amount of sulphuric acid added with the permanganate is less important. The amount of formaldehyde produced is greater with more acid, but, within the limits 0.1–0.3 c.c. the final results are indistinguishable, owing to the compensating effect of the higher final concentration of acid reducing the intensity of colour due to a definite quantity of formaldehyde. Quantities in excess of 0.5 c.c., however, might lead to formaldehyde being produced from ethyl alcohol itself.

"The time allowed for oxidation, provided it be not less than 3 minutes, appears to be without effect. The influence of temperature may not be negligible, but the point was not investigated, as this would clearly be constant in any one set of experiments.

"Not much less than 0.5 c.c. of a cold saturated solution of oxalic acid will reduce the excess of permanganate in the cold, faintly acid solution, but larger quantities, up to 1 c.c., appear to have no appreciable influence on the results.

"The subsequent addition of sulphuric acid must be as nearly as possible the same in any one set of experiments and is most conveniently made 1 c.c. as directed by Simmonds. With only 0.6 c.c. added, the acetaldehyde derived from pure ethyl alcohol will give a distinct colouration with Schiff's solution of the character described. With 0.75 c.c. or more, pure ethyl alcohol gives no purple or even blue colour, provided all the other standard conditions are rigidly adhered to, but it is unwise to reduce the amount below 1 c.c. except in very special circumstances, when it is necessary to make the test as sensitive as possible. In such cases, the sensitiveness of the test can be increased about 40% by using only 0.75 c.c. of acid, but very careful control experiments then become necessary. On the other hand, not more than 1 c.c. of acid should be used, as the use of so much as 1.25 c.c. reduces the sensitiveness of the test by about 30%, whilst 1.5 c.c. reduces it 50% and 2 c.c. nearly 90%.

"Finally, the amount of Schiff's solution taken is not without influence. If 10 c.c. be taken instead of 5 c.c., the concentration of acid is so much reduced that even the acetaldehyde from ethyl alcohol develops a colour. On the other hand, the use of only 2 c.c. in place of 5 c.c. reduces by about 90% the amount of colour developed by a fixed quantity of formaldehyde" (Jones, *Analyst*, 1915, 40, 218).

**United States Pharmacopœia Test.**—In this modification (U. S.

Pharmacopœia, IX, p. 36) of the fuchsin-bisulphite test, 5 c.c. of the alcohol diluted as in Simmond's test, are treated with 2 c.c. of a 3% potassium permanganate solution, 0.3 c.c. concentrated sulphuric acid added, the mixture allowed to stand for 5 minutes, the solution cleared by drop-wise addition of sulphurous acid with shaking, 1 c.c. of sulphuric acid and 5 c.c. of fuchsin-sulphurous acid test solution added, and mixed, and the solutions examined for bluish-violet colouration after 10 minutes. The fuchsin-sulphurous acid solution is made by dissolving 0.5 gm. fuchsin and 9 gm. of sodium bisulphite in 500 c.c. distilled water, and adding 10 c.c. of concentrated hydrochloric acid. The test will detect  $\frac{1}{4}$  to  $\frac{1}{3}$  of a part methyl per 100 parts of ethyl alcohol. Some objection has been made to it because of occasional false indications. The writer's experience with the method, however, has not been unfavourable, provided the test was carefully made.

**Chapin's Modification of Denigès' Test.**—Chapin (*J. Ind. Eng. Chem.*, 1921, 13, 543) has proposed replacing the U.S.P. test by the following modification of Denigès,' which is somewhat more sensitive than the U.S.P. test above. The liquid, purified as necessary (see purification precautions, p. 91) is diluted to a content of total alcohols equal to 5% by volume. To 5.0 c.c. add 0.3 c.c. of phosphoric acid (C.P. 85%), mix, add 2 c.c. of a 3% solution of potassium permanganate, mix and let stand until the permanganate is entirely decomposed (about 10 minutes). Add 1.0 c.c. of 10% oxalic acid, mix, and let stand until the colour becomes a clear brown (about 2 minutes). Add 1 c.c. conc. sulphuric acid, mix, add 5 c.c. of the Schiff reagent prepared according to Elvove's directions (below), immediately mix well and observe the colour after exactly 10 minutes. The solution may then possess a pale greenish tint, but should show no distinct blue or violet colour against a white background, indicating less than 0.2% methyl in the total alcohol. No attention should be paid to transitory acetaldehyde colour, developing quickly and then fading within 10 minutes. If due to acetaldehyde the colour will have disappeared 10 minutes after mixing. Variations in temperature between 15°–35° C. were reported by Chapin to be without influence on the results. Elvove's method (*J. Ind. Eng. Chem.*, 1917, 9, 295) for the preparation of Schiff's reagent is as follows: 0.2 gm. fuchsin is dissolved in 120 c.c. hot water. After cooling to room temperature 0.2 gm. anhydrous sodium sulphite

dissolved in 20 c.c. water is added, followed by 2 c.c. concentrated hydrochloric acid. The solution is diluted to 200 c.c. and allowed to stand for 1 hour before use. If well-stoppered in an amber bottle it may remain fit for use for several weeks.

**Modified Vorisek Test.**—Vorisek's chromic acid oxidation method (*J. Soc. Chem. Ind.*, 1909, **28**, 823) with gallic acid replacing albumin-ferric chloride as the formaldehyde reagent has given the writer good service. Gallic acid has the advantage of many formaldehyde reagents in giving a very positive colour. The frequent objection that it is too sensitive is met by following a fixed procedure and comparing with control tests on known mixtures. The test can be carried out in a shorter time than that of the U.S.P. above, and will detect about  $\frac{1}{3}$  part methyl in 100 parts ethyl alcohol.

Of the alcohol, or of distillate in case purification is necessary, 0.5 to 1.0 c.c. is put into a 6-in. test-tube; 1 c.c. of 0.8% solution of chromium trioxide (free from sulphuric acid) and water sufficient to make 5 c.c. are added. Two or three small pieces of pumice are dropped in and the tube attached to a simple air condenser made from a conveniently bent 4-ft. piece of glass tubing. The liquid is distilled at once into another test tube by boiling briskly over a small flame. The first 1 or 1.5 c.c. of distillate is collected separately and rejected. The distillation is carried to within 0.5 c.c. of dryness, and the distillate after mixing with 5 drops of a saturated alcoholic solution of gallic acid, is carefully underlaid with concentrated sulphuric acid. In the presence of even very small amounts of formaldehyde a clear blue ring develops at once or within a few minutes. Larger amounts of formaldehyde produce a dark green ring and acetaldehyde, for which the reagent is much less sensitive, a clear yellow to a red-brown, depending on the amount.

The distillate may be tested for formaldehyde by replacing the 5 drops of gallic acid solution with a like amount of a 0.5% solution of morphine sulphate, or better still a few milligrams of apomorphine. With either, formaldehyde gives a violet ring. Hinkel (*Analyst.*, 1908, **33**, 417) proposed morphine hydrochloride as the most delicate reagent but it has been pointed out since, that unreliable indications are obtained in the presence of chlorides. One should either use morphine sulphate or, if the hydrochloride is used follow the advice of Rabe (*Pharm. Ztg.*, 1921, **66**, 72; *C.A.*, 1923, **15**, 1373) and remove hydrochloric acid from the reagent by warming or allowing

to stand after mixing with sulphuric acid. Wolff (*Chem. Ztg.*, 1919, 43, 555; C.A., 1920, 14, 1504) finds that apomorphine gives a more characteristic colour with formaldehyde than morphine does, an observation confirmed by Gettler (*Jour. Biol. Chem.*, 1920, 61, 31).

**Identification by Crystalline Derivatives of Formaldehyde.**—Gettler recommends two methods for obtaining identifiable crystalline derivatives from the formaldehyde produced by the oxidation of methyl alcohol. The first is Mulliken's  $\beta$ -naphthol test (Mulliken, *Identification of Pure Organic Compounds*, I, 24). In this, 3 c.c. of the distillate from a suitably oxidised portion of the sample are placed in a test-tube, 0.04 to 0.06 gm. of  $\beta$ -naphthol and 3 to 5 drops of concentrated hydrochloric acid added. The mixture is boiled gently until the precipitate of methylene di- $\beta$ -naphthol appears in case formaldehyde is present. The fine white needles are filtered off, washed with 1:1 alcohol and dried. In a m. p. test in which the temperature is raised at the rate of  $1^\circ$  per 15 seconds as the m. p. is approached, the solid begins to turn brown at  $180^\circ$ , and melts with decomposition, giving a red-brown liquid, at  $189^\circ$ – $192^\circ$ , uncorrected.

In the second method (Romijn, *Chem. Zentr.*, 1895, ii, 257) 10 c.c. of the distillate after oxidation are evaporated just to dryness in a small dish on a water-bath, after 10 c.c. of strong ammonia water have been added. The residue is taken up in a few drops of water and a drop of this solution mixed on a microscope slide with a drop of mercuric chloride solution. Characteristic crystals of hexamethylenetetramine-mercuric chloride appear in case sufficient formaldehyde was present. The shape of the crystals is said to vary with the concentration; hence it is advised that here, also, parallel control tests be carried out on known mixtures of methyl and ethyl alcohols.

**Vivario Test.**—The hydrocyanic test proposed by Vivario (*J. pharm. chim.*, 1915, 10, 145; C.A., 1915, 9, 124) and recommended by Gettler is, like the Riche-Bardy test below, extremely useful for confirming indications obtained by the preceding tests, which all obviously depend on oxidation of the methyl alcohol to formaldehyde. The Vivario test is based on the fact that hydroxylamine in alkaline solution oxidizes methyl alcohol to formaldehyde; the latter, with the excess of hydroxylamine, gives formaldoxime,

which on heating decomposes to give hydrocyanic acid which is readily detected. Ethyl alcohol does not give the reaction. Formaldehyde and furfural if present should be removed by treatment with aniline phosphate or, preferably, with *m*-phenylenediamine hydrochloride, before applying the test. The procedure is as follows:

20 c.c. of the solution of mixed alcohols purified from aldehydes if necessary (and, according to Vivario, brought to about 80% total alcohol strength) are boiled for 7 hours in a reflux apparatus with 15 grm. potassium hydroxide and 1 grm. hydroxylamine hydrochloride. The mixture is then cooled, acidified with sulphuric acid, and distilled, the first (strongest in hydrocyanic acid) and two or three succeeding 5 c.c. portions being collected separately. The 5 c.c. portions of distillate are then tested by adding a few drops of sodium hydroxide solution, 2 or 3 drops of ferrous sulphate solution, and 2 or 3 drops of ferric chloride solution, then acidifying with hydrochloric acid. If hydrocyanic acid is present, Prussian blue is formed, and settles out on standing a few hours. In case so little is present that the result is doubtful, it is advisable to allow the solution to stand for a few hours, then pour through a small close-grained filter paper. By examination of the washed filter paper for blue colouration, one is able to detect the presence of a much smaller amount of Prussian blue, than by observation of the settled solution in a test-tube. The result may be confirmed by taking a second 5 c.c. portion, adding a few drops of sodium hydroxide, then 2 or 3 drops of yellow ammonium sulphide, and evaporating to dryness on the water bath. The residue is cooled, dissolved in a little hydrochloric acid, filtered, and the filtrate treated with 1 c.c. of ferric chloride solution. A red colour is produced in case the distillate contained hydrocyanic acid.

It is not necessary to adhere to Vivario's procedure of bringing the test solution to 80% total alcoholic strength, for with known mixtures in approximately 95% strength, tested as above, a positive result was obtained with 0.2 part methyl per 100 parts ethyl, and a doubtful result at 0.1 part. Furthermore Gettler applies the method, with satisfactory results, to solutions which must have been much weaker (perhaps as low as 20% although the exact strength was not stated).

**Riche-Bardy Test.**—This long-used method (Riche-Bardy, *Compt. rend.*, 1875, 80, 1076) tedious yet widely endorsed, depends on the  
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formation of methyl aniline violet. It is not quite so sensitive as the oxidation tests above but has an advantage over all except that of Vivario in this, that the dyed wool or floss is visible and permanent evidence of the finding. To make the test, 10 c.c. of the sample of alcohol, previously rectified if necessary over potassium carbonate, are placed in a small flask with 15 grm. iodine and 2 grm. red phosphorus. Methyl and ethyl iodides are formed and should be distilled off into about 30 c.c. of water. The heavy oily liquid which separates in the receiver is washed with dilute alkali to remove free iodine, separated and transferred to a flask containing 5 c.c. of aniline. The flask should be placed in cold water if the action is violent; or, if necessary, the reaction may be stimulated by gently warming the flask. After 1 hour the product is boiled with water and 20 c.c. of 10% sodium hydroxide added. The bases rise as an oily layer which may be drawn off with a pipette after filling the flask with water up to the neck. 1 c.c. of this oily liquid is oxidized by adding it to 10 grm. of a mixture of 100 parts of clean sand, 2 of common salt, and 3 of copper nitrate. After being thoroughly mixed, the mass is introduced into a glass tube and heated to 90° for 8 or 10 hours. The product is exhausted with warm alcohol, the liquid filtered and made up with alcohol to 100 c.c. If the sample of spirit was pure, the tint of the liquid is red, but in presence of 1% of methyl alcohol it has a distinct violet shade; with 2.5% the shade is very distinct and still more so with 5%. To detect more minute quantities of methyl alcohol, dilute 5 c.c. of the coloured liquid to 100 c.c. with water, and 5 c.c. of this again to 400 c.c. The liquid thus obtained is heated in porcelain, and a piece of undyed wool (white merino) 3 or 4 cm. square immersed in it. The fabric should be cleaned before use with warm soap suds, washed thoroughly and dried. It is stated that for this test the wool should be free from sulphur. It should be left in the liquid to be tested for about 30 minutes, then washed and dried. In certain of the U. S. Government laboratories at Washington uniform bits of silk floss are used with even better results. Pure alcohol will not produce a dye, but methylated alcohol will produce a violet, the depth of tint giving approximate indication of the proportion present. Comparison slips, made with 1, 2, 3 and 5% of methyl alcohol should be prepared as standards.

**Detection of Methyl Alcohol in Formalin.**—For detecting methyl alcohol, which is normally present, it is necessary to remove the

formaldehyde before applying any of the qualitative tests given above. Distillation with a slight excess of potassium cyanide (Leffmann, *Chem., Zent.*, 1905, **29**, 1086) with sodium sulphanilate, or with sodium phenyl hydrazine sulphonate have been used (see page 101). In any case the treatment should be repeated if necessary until no formaldehyde is found in the distillate.

**Estimation of Methyl Alcohol.**—When only methyl alcohol and water are present, the proportion of the former may be obtained from the sp. gr. by reference to the tables on page 89.

No general method for the estimation of methyl alcohol exists. It is possible only to describe methods which are applicable to the particular mixtures in which the analyst is most frequently called upon to estimate it. For example, in the assay of commercial wood naphtha it is usual to convert the methyl alcohol into methyl iodide and to estimate this. As all methoxy- and ethoxy-compounds yield volatile iodides under the conditions of the experiment, it is necessary to correct for the methyl acetate present in wood naphtha, and the method is clearly useless for mixtures of methyl and ethyl alcohols. On the other hand, the best method yet described for the estimation of methyl alcohol in admixture with ethyl alcohol is quite inapplicable to wood naphtha, since it depends on the oxidation of the methyl alcohol to carbon dioxide under conditions in which acetone and methyl acetate are similarly oxidized.

**Krell's Methyl Iodide Method.**—For the estimation of methyl alcohol in wood naphtha the following modification of Krell's method (*Ber.*, **6**, 1310, Also Gradski and Kramer, *Ber.*, 1873, **6**, 1492) is adopted in the British Government Laboratory:

"Twenty-two grams of coarsely-powdered iodine and 5 c.c. of distilled water are placed in a small flask and cooled by immersion in ice-cold water. Then 5 c.c. of the wood spirit (60.0° o. p. or about 91% by volume) are added, the flask corked, the contents gently shaken, and allowed to remain in the ice-cold bath for 10–15 minutes.

"When well cooled, 2 grm. of red phosphorus are added to the mixture of spirit and iodine in the flask, and the latter is immediately attached to a reflux condenser.

"The reaction soon commences, and must be moderated by dipping the flask into a cold water-bath. (Spirit may be lost if the reaction is too violent.) After about 15 to 20 minutes, when all action appears to have ceased, the water-bath under the flask is

gradually heated to a temperature of about 75° (167° F.), and the flask being occasionally shaken is allowed to remain at this temperature for 15 to 20 minutes. The source of heat is then removed and the apparatus left for an hour till it has cooled, when the condenser is reversed, and the methyl iodide is slowly distilled off—first at a low temperature—the bath being allowed to boil towards the end of the operation only. The end of the condenser dips into water in a measuring tube, and the iodide is collected under water and measured at a temperature of 60° F.

“The percentage by volume is found from the formula:

$$\frac{\text{c.c. methyl iodide found} \times 0.647 \times 100}{\text{c.c. wood spirit taken}} = \text{percentage of methyl alcohol}$$

“Or when 5 c.c. of spirit are taken:

$$\text{c.c. methyl iodide} \times 12.94 = \text{percentage by volume.}$$

“Esters and acetals also yield methyl iodide by this process, and from the percentage of methyl alcohol calculated as above an amount equivalent to the percentage of these substances present must be deducted. Practically, however, methyl acetate is the only compound usually found in quantity sufficient to materially affect the result. The number of grams of methyl acetate per 100 c.c. of spirit multiplied by 0.5405 gives the equivalent of methyl alcohol to be deducted from the total percentage by volume calculated from the methyl iodide found.”

The accuracy of the method is limited by the accuracy with which the 6 c.c. or so of methyl iodide can be measured. Methyl iodide is not quite insoluble in water, and it has been suggested that a correction for this solubility and for the vapour which remains in the apparatus should be made once for all by distilling 6 c.c. of pure methyl iodide and noting the deficiency from 6 c.c. of the distillate. On the other hand, some of the acetone which distils over remains dissolved in the methyl iodide; the volume so dissolved is small if the distillation be conducted as above described, but it does to some extent compensate for the loss of methyl iodide and perhaps renders unjustifiable any such refinement as the correction referred to.

Theoretically, all the sources of error enumerated above would be avoided by having recourse to Zeisel's method (*Monatsh. fur. Chem.*, 6, 989; 7, 406) for the estimation of methoxy-groups, in which the whole of the methyl iodide vapour is swept out of the apparatus

by a current of carbon dioxide and decomposed by alcoholic silver nitrate solution, yielding silver iodide which can be weighed with any degree of accuracy desired. Stritar and Zeidler (*Zeit. anal. Chem.*, 1904, **43**, 387) have advocated this procedure and have suggested some simplification of Zeisel's apparatus. Duplicate analyses, however, differ by 1%; furthermore, other methyl compounds such as methyl acetate and methyl acetal are usually involved with no adequate method for correcting for them. Hence for crude wood naphthas at least this tedious gravimetric method has little cause to be preferred on the score of accuracy to the simpler volumetric one.

**Estimation of Methyl Alcohol in Formaldehyde Solutions.**—

Methods for this purpose fall into two groups: (a) those in which the formaldehyde solution is distilled after adding a reagent to retain the formaldehyde and the methyl alcohol estimated in the distillate, usually from the sp. gr.; (b) those in which the formaldehyde is determined separately, and the methyl alcohol estimated from the amount of standard potassium bichromate or permanganate solution required for its oxidation. From the total amount of oxidizing agent consumed is deducted the amount corresponding to the formaldehyde found by analysis, the difference being taken as the amount used by the methyl alcohol.

For all methods of group (a), a large quantity of reagent is required and the best, sodium phenylhydrazine sulphonate, is costly. Others which have been used are sodium sulphanilate (Gnehm and Kaufler, *Zeit. angew. chem.*, 1904, **19**, 673); ammonia (Duyk, *Ann. chim. anal.*, 1901, **1**, 407); sodium bisulphite (Bamberger, *Zeit. anorg. Chem.*, **17**, 1246); and calcium saccharate (Orloff, *Formaldehyde*, 1909, Kietaihl's German translation p. 216). Gnehm and Kaufler's method is as follows: 90 grm. of sodium sulphanilate are added gradually to 25 c.c. of water which is kept boiling until all is dissolved. The flask containing the mixture is rapidly cooled, the contents being stirred all the time with a rod. To the crystalline mass, 20 c.c. of the formalin is added, the flask corked and left for 3 or 4 hours. The mixture is next submitted to distillation and the first 35 c.c. of the distillate collected. This is diluted to 50 c.c. and its sp. gr. taken.

In group (b), the method of Lockemann and Kroner (*Zeit. anal. Chem.*, 1895, **34**, 623) makes use of Hetper's oxidation with potassium permanganate (*Z. Nahr. Genussm.*, **24**, 731, **26**, 342) the formaldehyde in the original being determined preferably by the hydroxy-

lamine method (Brochet and Cambier, *Z. anal. Chem.*, 1895, **34**, 623. (See Determination of Acetone) or, in case iron is present, by Lemme's (*Chem. Ztg.*, 1903, **27**, 896, Cf. Doby, *Z. angew. Chem.*, **20**, 353) neutral sodium sulphite method. The older method of Blank and Finkenbeiner (*Ber.*, 1906, **39**, 1327) uses bichromate oxidation and is carried out as follows: 1 grm. of the formalin is mixed with 50 c.c. of 2.0 N chromic acid (66.68 grm. per litre), 20 c.c. pure concentrated sulphuric acid added, the mixture allowed to stand 12 hours, and then made to 1000 c.c. To 50 c.c. of the diluted solution a small crystal of potassium iodide is added and the solution titrated back with N/10 sodium thiosulphate. The whole process can be completed in 2 hours if the oxidation be assisted by warming after the first violent reaction is over, but great care is necessary and the solution must not be evaporated below two-thirds its original volume.

If exactly 2.0 N chromic acid was used, 1 grm. sample, known to contain 40% formaldehyde, taken, and "a" c.c. N/10 sodium thiosulphate used in the titration, then:

$$\frac{2 \times 10 \times 50}{20} - a = b \text{ c.c. N/10 solution used for both;}$$

$$b - \left( \frac{0.40}{20 \times 0.00075} \right) = c \text{ c.c. N/10 used for the methyl alcohol;}$$

$$\text{and } \frac{20 \times c \times 0.000534}{100} = \% \text{ methyl alcohol in the sample.}$$

### Estimation of Methyl Alcohol in Presence of Ethyl Alcohol.

**Thorpe-Holmes Method:** This is probably the best chemical method available. It is accurate, occupies but little of the analyst's time, and can be conducted in any laboratory. The refractometer method described below is more rapid and in certain concentrations quite as accurate, but it depends on the use of an instrument which is not to be found in every laboratory. The method of Thorpe and Holmes (*Trans. Chem. Soc.*, 1904, **85**, 1) depends on the complete oxidation of methyl alcohol to carbon dioxide by means of chromic acid mixture. Ethyl alcohol under the conditions of the experiment yields carbon dioxide equivalent to approximately 0.5% of its weight. (For information as to variation to be expected in this factor, see Wilks, Sudan Gov't Welcome Tropical Research Lab. Khartoum, Chem. Section, *Bull.* I, October, 1914; C.A., 1915, **9**, 2205; also Koenig (*Chem. Ztg.*, 1912, **36**, 1025). The process is as follows:

"The sample is mixed with water in such proportions that 50 c.c. of the mixture shall contain not more than 1 gram. of methyl alcohol and in the presence of ethyl alcohol, not more than 4 gram. of the mixed alcohols. 50 c.c. of this mixture are then introduced into a 300 c.c. flask, which can be closed by a ground-in stopper and which is fitted with a funnel and a side tube. 20 gram. of potassium bichromate and 80 c.c. of dilute sulphuric acid (1:4) are added, and the mixture allowed to remain 18 hours. A further quantity of 10 gram. of potassium bichromate and 50 c.c. of sulphuric acid mixed with an equal volume of water are now added, and the contents of the flask heated to the b. p. for about 10 minutes, the evolved carbon dioxide being swept out of the apparatus by a current of air and collected in soda lime.

"When ethyl alcohol is present, a subtractive correction must be applied to the weight of the carbon dioxide thus obtained in the proportion of 0.01 gram. of carbon dioxide for each gram of ethyl alcohol present."

As in most cases the liquid to be examined will contain at least 10 times as much ethyl alcohol as methyl alcohol, the total alcoholic content may be determined with sufficient accuracy from the sp. gr. by reference to the ethyl alcohol tables.

In the above-described oxidation process acetone and methyl alcohol are converted into acetic acid and carbon dioxide, while allyl alcohol is wholly oxidized yielding carbon dioxide. As the proportion of these substances in wood naphtha used in Great Britain for methylating (denaturing) is fairly constant, and as none of them are normal constituents of commercial ethyl alcohol, the fact that they take part in the reaction is of less importance where the object is to detect methylated spirit in tinctures or to estimate the proportion of wood naphtha in a sample of methylated spirit. The bulk of the secondary constituents of wood naphtha may be removed from the spirit by shaking with light petroleum and saturated salt solution; the alcohols are then recovered from the saline layer by distillation and submitted to the oxidation process. Even with this treatment the methyl alcohol will commonly be overestimated by 4%, that is to say, 5.2% will be found where only 5% is present.

Frequently it is more convenient and equally satisfactory to replace the gravimetric procedure of Thorpe and Holmes by a volumetric one. Absorption of the carbon dioxide in barium hydroxide

solution in a Meyer sulphur tube, filtration of the barium carbonate, followed by titration with standard acid using methyl orange indicator, according to the carbon dioxide method of Cain and Maxwell (*J. Ind. Eng. Chem.*, 1918, 10, 520) has been found to give satisfactory results.

For the **estimation of methylated spirit in tinctures**, Thorpe and Holmes recommend that the spirit from 25 c.c. of the sample, or from 50 c.c. if it contains less than 50% of alcohol, be treated with light petroleum to remove essential oils, etc., as described in a later subsection (estimation of alcohol in essences) and then distilled and diluted with water to a volume of 250 c.c.; 50 c.c. of this mixture is then oxidized with chromic acid mixture as described above. If the weight of carbon dioxide thus obtained does not exceed 0.01 grm. for each gram of alcohol present, this amount being equivalent to 0.7 vol. of methyl alcohol in 100 vols. of the alcohol, then it may be concluded that the sample contains only spirits of wine. Should the amount of carbon dioxide exceed this, its equivalent in methyl alcohol by volume must be subjected to a subtractive correction of from 0.7 to 1% (depending on the amount of methylated spirit present), the percentage of methylated spirit being calculated on the assumption that the quantity of methyl alcohol occurring in dehydrated methylated spirit does not exceed 8.8%.

**Hetper's Permanganate Method.**—Hetper (*Z. Nahr. Genussm.*, 24, 6731; 26, 343; C.A., 1913, 7, 860; 1914, 8, 479) after a study of the oxidation of very dilute ethyl and methyl alcohol solutions by potassium permanganate, proposed a method unique in that *both* alcohols are determined with accuracy in mixtures containing as little as 0.1% by weight. The method is based on the following facts: (1) In an acid solution (phosphoric) methyl alcohol is oxidized by potassium permanganate to carbon dioxide and water, and ethyl alcohol to acetic acid and water. (2) In an alkaline solution, methyl alcohol is oxidized to carbon dioxide and water as before, but ethyl is oxidized to a mixture of acetic and oxalic acids, the proportion depending on conditions of temperature, etc. By controlling the excess of permanganate, the temperature, and the dilution of the solution, ethyl alcohol can be made to reduce the same amount of permanganate as an equal weight of methyl alcohol. The method, if it proves as useful in the hands of others, will take care of a class of cases for which no method has been available,

namely, where the alcohol content is very small, and the amount of sample insufficient to permit concentrating to strength at which the other methods become usable.

**Immersion Refractometer Method.**—This method of Leach and (Lythgoe, *J. Am. Chem. Soc.*, 1905, **27**, 964), widely used both for detection and for estimation of methyl alcohol in the presence of ethyl, is based on the use of the Zeiss immersion refractometer, and when this instrument is available it affords a most rapid means of estimating the two alcohols. With the immersion refractometer at 20°, distilled water gives a reading of 14.5 scale divisions. Addition of ethyl alcohol increases the reading, until at about 75% alcohol a maximum of 101 divisions is reached; further addition of alcohol reduces the reading until at 100% alcohol it has fallen to 91. Small additions of methyl alcohol to water also increase the readings, but to a lesser degree, and a maximum is reached at 50% alcohol when the reading is 39.8; further addition of methyl alcohol reduces the reading, so that at 91% it is again 14.9 or about the same as pure water, while at 100% alcohol the reading is only two divisions. Leach and Lythgoe give two tables, of which one is here reproduced, and an example of its use follows. The table shows the reading of the immersion refractometer corresponding to each percentage of alcohol, both ethyl and methyl, by weight, all readings being taken at exactly 20°. This table will show at a glance whether a solution of given strength of alcohol, as determined from the sp. gr., contains ethyl or methyl alcohol or is a mixture of the two.

For the estimation of methylated spirit in tinctures and essences it is necessary to obtain the alcohols free from non-volatile matters and from essential oils before subjecting them to refractometric treatment. The method of Thorpe and Holmes (see "Estimation of Alcohol in Essences and Tinctures"), in which the tincture is mixed with salt solution and the oils, etc. extracted with light petroleum, is preferable, as this treatment removes most of the acetone as well. By either of these methods the estimation of methyl alcohol is combined with that of ethyl alcohol and requires that only a refractometric reading be made on the distillate, the sp. gr. of which has been taken to ascertain the total alcoholic strength.

Where it is required to estimate small amounts of methyl alcohol by the refractometer, it is well to redistil the distillate and collect only the first 50 c.c., or even 25 c.c., thereby obtaining a liquid

sufficiently rich in alcohol so that the readings fall in that portion of the table where the two alcohols differ widely. In such distillations great care must be used each time to drive all the alcohol over into the distillate if the ratio of methyl to ethyl alcohol is to remain unchanged. The behavior of a mixture of these two alcohols on fractionation depends on whether there is much or little water present. For example, with a 95% ethyl alcohol containing a little methyl (0.2%), the latter tends to concentrate in the distillate. When the same mixture is diluted with water to 50% or less and then fractionated, the first alcohol over is *weaker*, and the very last alcohol to leave the solution is proportionately *much richer*, in methyl alcohol than the original. Furthermore, when a diluted methyl alcohol containing a little ethyl alcohol is distilled, the latter tends to concentrate in the *first portions over*. Failure to take into account these facts may lead to false conclusions, especially when one is looking for methyl alcohol in the presence of a large amount of ethyl, and distillation has to be resorted to, either to purify or to concentrate the alcohols.

*Example.*—An orange extract was diluted four times with water, treated with magnesia and filtered. A measured portion of the filtrate was then distilled and the distillate made up to the measured portion taken. This distillate was found to have a sp. gr. of 0.9754 corresponding to 16.91% by weight, and to have a refraction of 42.0 on the Zeiss immersion refractometer. By interpolation in the table, the readings of ethyl and methyl alcohol corresponding to 16.91% alcohol are 44.3 and 24.45 respectively, the difference being 19.85.  $44.3 - 42.0 = 2.3$ .  $100(2.3 - 19.85) = 11.6$ . Thus 11.6% of the alcohol present was methyl alcohol.

Recently Lange and Reif, *Z. Nahr. Genussm.*, 1921, 41, 217) have proposed a better method in which the distillate is either concentrated or diluted, as necessary to bring to 50% alcohol by volume (sp. gr. 0.9346), the refractometer reading taken, and the per cent. methyl alcohol found by reference to a table giving Zeiss immersion refractometer readings for the whole range of ethyl alcohol-methyl alcohol mixture containing 50 vol. % of total alcohols. A table is given also, showing for each 0.1% above 50 vol. %, the volume of water which must be added per 100 c.c. of distillate in order to obtain a diluted alcohol of the required sp. gr.

SCALE READINGS ON ZEISS IMMERSION REFRACTOMETER AT 20°  
Corresponding to each % by weight of Ethyl and Methyl Alcohol

Per cent. alcohol by weight	Scale readings		Per cent. alcohol by weight	Scale readings	
	Methyl alcohol	Ethyl alcohol		Methyl alcohol	Ethyl alcohol
0	14.5	14.5	50	39.8	90.3
1	.8	16.0	51	.7	91.1
2	15.4	17.6	52	.6	.8
3	16.0	19.1	53	.6	92.4
4	.6	20.7	54	.5	93.0
5	17.2	22.3	55	.4	.6
6	.8	24.1	56	.2	94.1
7	18.4	25.9	57	.0	.7
8	19.0	27.8	58	38.6	95.2
9	.6	29.6	59	.3	.7
10	20.2	31.4	60	37.9	96.2
11	.8	33.2	61	.5	.7
12	21.4	35.0	62	.0	97.1
13	22.0	36.9	63	36.5	.5
14	.6	38.7	64	.0	98.0
15	23.2	40.5	65	35.5	.3
16	.9	42.5	66	.0	.7
17	24.5	44.5	67	34.5	99.1
18	25.4	46.5	68	.0	.4
19	.8	48.5	69	33.5	.7
20	26.5	50.5	70	.0	100.0
21	27.1	52.4	71	32.3	.2
22	.8	54.3	72	31.7	.4
23	28.4	56.3	73	.1	.6
24	29.1	58.2	74	30.4	.8
25	29.7	60.1	75	29.7	101.0
26	30.3	61.9	76	.0	.0
27	.9	63.7	77	28.3	100.9
28	31.6	65.5	78	27.6	.8
29	32.2	67.2	79	26.8	.8
30	.8	69.0	80	.0	.7
31	33.5	70.4	81	25.1	.6
32	34.1	71.7	82	24.3	.5
33	.7	73.1	83	23.6	.4
34	35.2	74.4	84	22.8	.3
35	.8	75.8	85	21.8	.1
36	36.3	76.9	86	20.8	99.8
37	.8	78.0	87	19.7	.5
38	37.3	79.1	88	18.6	.2
39	.7	80.2	89	17.3	98.9
40	38.1	81.3	90	16.1	.6
41	.4	82.3	91	14.9	.3
42	.8	83.3	92	13.7	97.8
43	39.2	84.2	93	12.4	.2
44	.3	85.2	94	11.0	96.4
45	.4	86.2	95	9.6	95.7
46	.5	87.0	96	8.2	94.9
47	.6	.8	97	6.7	.0
48	.7	88.7	98	5.1	93.0
49	.8	89.5	99	3.5	92.0
			100	2.0	91.0

### Wood Naphtha—Crude Wood Spirit

In addition to methyl alcohol and water, commercial wood naphtha contains acetone and higher ketones (nil to 14%), esters, mainly methyl acetate (nil to 4%), together with smaller proportions of acetaldehyde, dimethylacetal, allyl alcohol, pyridine, aliphatic

amines, and other substances. Klar (*Technol. der Holzverkohlung*, 1909, 208) gives the following as the approximate composition of American crude wood spirit (80 vol. %, 73.5% by weight):

55-50% methyl alcohol.

12-14% acetone.

5-10% aldehyde, methyl acetate, amines, higher ketones, allyl alcohol and wood oil, etc.

28-26% water.

For use in the colour industry a very pure spirit is required, acetone being a highly objectionable impurity, and as a consequence an acetone-free grade is now a regular article of commerce. For dissolving resins to make varnishes, the presence of acetone is an advantage on account of its solvent properties. For the complete denaturing of ethyl alcohol, the proportion of acetone and of nauseous constituents—allyl alcohol, pyridine, etc.—is most important.

### Analysis of Wood Naphtha

The analytical requirements of the producer of crude wood spirit are very simple. The composition of the normal product of the operation of his plant has been more or less thoroughly learned from experience. A determination of sp. gr. with reference to methyl alcohol (or ethyl alcohol) tables supplies the desired information as to quantity. In addition, only such qualitative tests as observation of colour, miscibility with water, proportion insoluble in 38° Bé. sodium hydroxide solution (acetone), etc., are usually needed. The purchaser's requirements on the other hand are not so simple. Not knowing the history of the product, he requires an examination which will ensure the detection of an abnormal mixture, especially one with too high a proportion of low-grade, methyl alcohol-poor, products of refining. The methods which have come to be used for this purpose are essentially the same in different countries and usually include estimation of the following: (1) methyl alcohol, (2) acetone (3) methyl acetate, (4) allyl alcohol, and (5) ammonia, amines, pyridine, etc.

The methods now to be described are applicable not only to crude or partially purified naphtha, but to any commercial sample of wood spirit. The impurities to be looked for and estimated are the same in every grade of spirit up to the so-called acetone-free grade; the vari-

ous grades differ only in the amounts of the secondary constituents, with the reservation that some crude, usually high-coloured, naphtha contain impurities of high b. p. (150–200° and over), hydrocarbons, acids and bases, which are scarcely detectable in spirit of average quality. Unless otherwise stated, the methods detailed below are those of the British Government Laboratory for the examination of wood naphtha.

1. *Estimation of Methyl Alcohol*.—The British method has been given in an earlier subsection (p. 99).

2. *Estimation of Acetone and Higher Ketones*.—These are usually estimated by Messingers' (*Ber.*, 1888, 21, 3366) method and calculated as acetone. The method is rapid and with solutions of pure acetone in water or methyl alcohol gives excellent results. Applied to wood naphtha it is less satisfactory; the whole of the acetone reacts no doubt, so that the number obtained is not less than the amount of real acetone present. Certain higher ketones, which are present, also take part in the reaction, but reduce a smaller amount of iodine per unit of weight than does acetone, so that the number obtained is lower than the true amount of ketones present. Denigès' method (*J. de pharm. et de chim.*, 1899, 9, 7; *Bull. soc. chim.*, 1899, 19, 754) based upon the formation of an insoluble compound of acetone with mercuric sulphate, is sometimes used but is open to similar objection and occupies more time. The hydroxylamine method (see p. 124) seems to have been but little used in this connection, but according to the writer's experience it deserves greater consideration. Messinger's method is followed with but slight modification in the British Government Laboratory, where the following procedure is adopted:

25 c.c. of N/1 sodium hydroxide are placed in a stoppered flask of about 200 c.c. capacity. To this is added 0.5 c.c. of the naphtha. The mixture is well shaken and allowed to stand 5 to 10 minutes. Into it from a burette N/5 iodine solution is run slowly, drop by drop, vigorously shaking all the time, till the upper portion of the solution on standing a minute becomes quite clear. A few c.c. more of N/5 iodine solution are added, since to get concordant results an excess of at least 25% of the iodine required must be added. After shaking, the mixture is allowed to stand for 10 to 15 minutes, and then 25 c.c. N/1 sulphuric acid are added. The excess of iodine is liberated, titrated with N/10 sodium thiosulphate solution and

starch, and half the number of c.c. of thiosulphate solution used are deducted from the total number of c.c. of iodine solution used. The difference gives the amount of acetone by weight in the naphtha by the formula: c.c. N/5 iodine solution required  $\times 0.387 =$  grm. acetone per 100 c.c. of wood naphtha.

"This includes as acetone any aldehydes, etc., capable of yielding iodoform by this reaction.

"If the quantity 'of acetone' is excessive, a less quantity of the spirit is taken, or 10 c.c. are diluted with 10 c.c. of methyl alcohol free from acetone, and 0.5 c.c. of the mixture is used."

Messinger in his original communication calls attention to the fact that commercial sodium hydroxide may contain nitrite, which must be allowed for. This is easily done by making a blank test, in which only the naphtha is omitted. This precaution is advisable even though there is little danger of finding sodium hydroxide with nitrite impurity.

The procedure given above is sufficiently accurate for the examination of wood naphtha when the nature of the latter is considered. Sometimes, as in the examination of commercial acetones, greater consistency is desired. In such case it is advisable to put the sample into a weighed thin-walled glass bulb, which is then sealed, weighed and broken under the surface of a relatively large volume of water in a graduated flask. The solution is diluted to the mark and thereafter handled in such a way as to avoid as far as possible volatilization of the acetone. The titration is carried out according to Goodwin's modification (*J. Am. Chem. Soc.*, 1920, **42**, 39), of the Messinger method, as follows:

An aliquot, containing 30 to 40 mg. of acetone is added to 50 c.c. of N/1 sodium hydroxide in a glass-stoppered container. After 5 minutes standing, about 25% excess of N/10 iodine solution is run in from a burette with continual shaking. The bottle is stoppered and the solution allowed to stand for 10 minutes (20 in cold weather). 25 c.c. of 2.0 N sulphuric acid are added from a measuring cylinder, care being taken to add only 0.3 to 0.4 c.c. in excess of the amount required to neutralize the alkali. The liberated iodine is then titrated with N/20 sodium thiosulphate solution, fresh starch indicator being added near the end of the titration. 1 c.c. of N/10 iodine solution is equal to 0.0009675 grm. of acetone.

3. *Estimation of Esters*.—To 5 c.c. of the naphtha contained in a

small Jena glass flask, 20 c.c. of recently boiled distilled water are added, and then 10 c.c. of N/1 sodium hydroxide and the whole heated for 2 hours under a reflux condenser on the water-bath. The liquid is then cooled, phenolphthalein added, and the excess of sodium hydroxide titrated with N/1 acid.

Let the amount of acid required be  $x$  c.c. Then the number of grams of esters (calculated as methyl acetate) in 100 c.c. of the sample, is  $1.48(10 - x)$ . If the proportion of esters is very small and it is required to estimate it with great accuracy, a much larger quantity of spirit may be taken, and N/10 alkali and acid used, but in this case the spirit should be first boiled under a reflux condenser to expel carbon dioxide. Wood spirit is generally almost neutral to phenolphthalein, but if not it must of course be rendered neutral before proceeding to the estimation of esters. In the British Government Laboratory the hydrolysis of the esters is conveniently effected in a silver pressure flask of about 150 c.c. capacity. The writer, however, prefers to make these saponifications in the cold, the sample (weighed in sealed bulbs if of high esters content) being allowed to stand over night in pressure bottles ("citrate" bottles) containing an excess of standard alkali solution. The ester is easily saponified and working in this way there is not enough colour produced from the resinification of acetaldehyde, in case much of the latter is present to interfere with the titration.

4. *Estimation of Unsaturated Compounds (Allyl Alcohol).*—No accurate method for the estimation of allyl alcohol in wood naphtha exists, but the amount of bromine which the naphtha will decolourize is some measure of the unsaturated compounds, of which allyl alcohol is known to be the one commonly present. For denaturing spirits of wine in Britain, wood naphtha is required to have a certain minimum capacity for decolourizing bromine; not more than 30 c.c. of the naphtha must be necessary to decolourize 0.5 gram. of bromine. The test is conducted as follows:

A standard bromine solution is made by dissolving 12.406 gram. of potassium bromide and 3.481 gram. of potassium bromate in a litre of recently boiled distilled water.

50 c.c. of this standard solution (=0.5 gram. bromine) are placed in a flask of about 200 c.c. capacity, having a well-ground glass stopper. To this is added 10 c.c. of dilute sulphuric acid (1:4) and the whole shaken gently. After standing for a few minutes,

the wood naphtha is slowly run in from a burette into the clear brown solution of bromine until the latter is completely decolorized. The allyl alcohol content of crude wood spirit is 0.3 to 0.5%. The wood spirit used for denaturing has a somewhat higher allyl content, the latter having been increased by the addition of those fractions from the refining which are high in allyl alcohol.

5. *Estimation of Basic Substances*.—These (pyridines, mono-, di-, and trimethylamine) can be to some extent measured by the methyl orange alkalinity of the sample, though as it is not known which base predominates it is not usual to calculate the bases, as pyridine for instance, in the same manner in which ketones are calculated as acetone and the esters as methyl acetate. Fawsitt, to whom we owe much of our information concerning wood alcohol, mentions the methylamines, but not pyridine, which is certainly present in most samples. But no doubt the bases in naphthas differ with the source of the naphtha. For use as a denaturant in Britain the wood naphtha must comply with the following specifications as to reaction with indicators.

"The naphtha should be faintly acid to phenolphthalein, slightly alkaline or neutral, rarely acid to litmus, and always alkaline to methyl orange. 25 c.c. of the wood naphtha are placed in each of two beakers, and titrated with N/10 acid, using in one case a few drops of litmus solution, and in the other a solution of methyl orange as indicator. With litmus usually 0.1 to 0.2 c.c. of N/10 acid is required to neutralize. With methyl orange the total alkalinity should be greater, at least 5 or 6 c.c. of N/10 acid being required for neutralization.

"The total alkalinity, less that given with litmus is the methyl orange alkalinity and, for the 25 c.c. of wood spirit, should not be less than is required to neutralize 5 c.c. of N/10 acid."

**Miscellaneous Tests for Wood Naphtha**.—*Furfural* may be detected by adding 10 c.c. of the naphtha to 1 or 2 c.c. of acetic acid in which a few drops of aniline have been dissolved. Furfural if present will develop an intense red, but as the acetic acid itself may contain furfural the acid and aniline must be mixed first and must remain colourless for five minutes before the spirit is added.

**Wood Oil and Tar**.—Wood spirit occasionally contains 1% or more of substances of high b. p. (150°–200° and over) which may be separated simply by slow distillation of a large quantity on the water

bath. As the residue is tar, the method to be adopted for its further examination must be looked for in another section. This tar has the characteristic offensive odour of crude naphtha, only in a greater degree. Separated into hydrocarbons, phenolic bodies and bases, only the latter are found to be offensive; the hydrocarbons have the odour of terpenes, while the predominant phenolic body is no doubt guaiacol, which, alone, has a grateful odour, but in combination with pyridine bases, it appears to make their offensive odour even more offensive.

*Ethyl Alcohol*.—The detection of small admixtures of ethyl alcohol in wood spirit is less important than the converse. The following tests have been proposed for the purpose.

Berthelot suggested heating the sample with twice its volume of concentrated sulphuric acid. If 1% of ethyl alcohol is present, ethylene is evolved, and may be absorbed by bromine and estimated as ethylene di-bromide. Acetone and the normal impurities of wood spirit may yield carbon monoxide and dioxide, but not ethylene.

Riche and Bardy (*Comp. rend.*, 1876, **82**, 768) use a reaction depending on the production of aldehyde from ethyl alcohol by oxidizing agents, and the action of aldehyde, methylacetal, etc., on salts of rosaniline, whereby a violet colouring matter is produced which is not destroyed by subsequent addition of sulphurous acid. 4 c.c. of the liquid to be examined are mixed with 6 c.c. of conc. sulphuric acid and 10 c.c. of water. 7 or 8 c.c. are distilled into 10 c.c. of water and to this liquid are added 5 c.c. of sulphuric acid and 10 c.c. of a solution of potassium permanganate of 1.028 sp. gr. After 5 minutes have elapsed, 4 c.c. of a solution of sodium thio-sulphate, of 1.29 sp. gr., and 4 c.c. of a solution of magenta, containing 0.02 gm. per 1000 c.c. are added. Under these conditions, wood spirit unmixed with ethyl alcohol gives a yellowish-white liquid, but if ethyl alcohol is present the solution assumes a violet colour of greater or less intensity. Acetone, formic acid, and isopropyl alcohol give no similar reaction.

*Aldehyde (Dimethylacetal)*.—A determination of this normal impurity in crude wood naphtha and the lower products of methyl alcohol refining is occasionally required, but no satisfactory method has been proposed. Some use has been made of the following, based on Romijn's cyanide method (*Z. anal. Chem.*, 1897, **36**, 21.)

25 c.c. of the spirit is diluted to about 200 c.c. with water, and, after adding 1 c.c. of concentrated sulphuric acid to decompose the acetal, is heated so as to distil *very* slowly about one-half the liquid through a 15 disc Young's column. The distillate, collected in an ice-cooled receiver, is diluted to a convenient volume. An aliquot containing about 0.04 grm. acetaldehyde is then shaken in a closed flask containing 25 c.c. of potassium cyanide solution (6.2 to 7.0 grm. per litre according to cyanide content). This mixture is then poured into a 200 c.c. volumetric flask containing 30 c.c. of N/10 silver nitrate, acidified with a few drops of concentrated nitric acid. After mixing, the solution is diluted to the mark, mixed thoroughly and poured on a dry filter. 100 c.c. of the clear filtrate is titrated with N/10 potassium sulphocyanate, using 5 c.c. of saturated ferric alum solution as indicator. A blank test is made in exactly the same way except that the portion of distillate is omitted. If  $a$  is the number of c.c. of N/10 sulphocyanate solution obtained by subtracting the blank from the c.c. used in the test, then  $2 \times a \times 0.0044$  gives the grams acetaldehyde in the portion taken for the titration.

If the acetaldehyde is completely depolymerized, the cyanide method serves very well for its determination. Acetone has not been found to interfere seriously. The result obtained represents not only the acetaldehyde which was present initially in the free state but also that present in the form of paraldehyde, and of dimethylacetal. Unless odour and other qualitative tests show considerable free acetaldehyde in the original this result is best calculated to dimethylacetal, which may be present to the extent of 1 to 2% in the crude spirit. The determination of the free acetaldehyde by this method is not so satisfactory, apparently because of the interference of certain impurities, presumably basic, which the distillation with sulphuric acid removes.

*Wood Alcohol for Denaturing.*—The specifications with which wood spirits, intended for completely denaturing ethyl alcohol, must comply differ considerably in Great Britain and the United States. In Great Britain the wood naphtha must be sufficiently impure to impart to the methylated spirit such an amount of nauseousness as will, in the opinion of the Principal of the Government Laboratory render such a mixture incapable of being used as a beverage or of being mixed with potable spirits of any kind without rendering them

unfit for human consumption. It must conform to the following tests:

(a) Not more than 30 c.c. should be required to decolourize a solution containing 0.5 grm. of bromine.

(b) It should be neutral or only slightly alkaline to litmus, and 25 c.c. should require at least 5 c.c. of N/10 acid when methyl orange is used as indicator.

It should contain:

(a) At least 72% by volume of methyl alcohol.

(b) Not more than 12 grm. per 100 c.c. of acetone, aldehydes, and higher ketones, estimated as "acetone" by Messinger's method.

(c) Not more than 3 grm. per 100 c.c. of esters, estimated as methyl acetate by hydrolysis.

In the United States the colour must not exceed that of freshly prepared 0.0002 N iodine solution, the sp. gr. must not exceed 0.830 at 60°/60° F., and 90% should distil below 75° at 760 mm. in a specified apparatus. Diluted with a double volume of water, the spirit should remain clear or develop only a slight opalescence. The "acetone" by Messinger's method must not be less than 15 nor more than 25 grm. per 100 c.c. and the esters, calculated as methyl acetate must not exceed 5 grm. per 100 c.c. Not less than 15, no more than 25 c.c. should be required to decolourize a solution containing 0.5 grm. of bromine. In addition the wood alcohol must be of such a character as to render the ethyl alcohol with which it is mixed unfit for use as a beverage. These specifications apply to the wood spirit which is to be used as a so-called "complete denaturant." For certain of the specially denatured alcohols (S. D. Formulæ 2, 3, 3a, 6, 14, 16, 30), "commercially pure" methyl alcohol having a sp. gr. of not more than 0.810 at 60°/60° F. is required.

The requirements of various other countries for crude wood spirit to be used as a denaturant are given in Klar's *Technologie der Holzverkohlung*, 2 Auflage, pp. 369-382 (1909, also reprint of 1921).

### Commercial and Refined Methyl Alcohol

If the refining of crude wood spirit by the continuous process involved a water solution containing only acetone and methyl alcohol, the products would be pure methyl alcohol and the constant-boiling mixture of methyl alcohol and acetone containing 85-88% of the

latter. However, the proportion of other constituents is considerable, and, in addition, methods of refining are not uniform, so that the products which appear in the American market vary greatly, as the following list of commercial grades shows:

1. *Methyl Acetone*.—Sp. gr. 0.83–0.85, contains 45–55% of acetone (Messinger or hydroxylamine methods), 15–25% of methyl acetate, and 15–45 c.c. are required to decolourize 0.5 grm. bromine.

2. *Denaturing Spirit*.—Sp. gr. not over 0.830 and otherwise of composition fixed by Government requirements given above.

3. 95% *Methanol*.—Sp. gr. about 0.815 (95° Tralles), containing 20–30% of acetone, and about 5% of methyl acetate.

4. 97% *Methanol*.—Sp. gr. about 0.807–.808 (97° Tralles), containing 10–20% acetone, and 1–3% methyl acetate.

5. *Commercially Pure Methyl Alcohol (Columbian Methanol)*.—Practically pure methyl alcohol, containing a little water, sp. gr. not over 0.799 at 60°/60° (99° Tralles), and containing not over 0.1% acetone. This grade is used for the manufacture of formaldehyde and dimethylaniline.

6. *Reagent Grade, or Refined Chemical Methyl Alcohol*.—This is a product which has been subjected to additional refining, to reduce in particular the content of acetone. The alcohol should be neutral to both phenolphthalein and methyl orange, free from residue, should not decolourize bromine, and when shaken with sodium hydroxide solution (1.3 sp. gr.) should not develop any colour. On mixing 10 c.c. with an equal volume of alcoholic 10% solution of potassium hydroxide solution, adding 2 drops of aniline, and boiling, no odour of isonitrile should develop, indicating the absence of chloroform.

The strength of the alcohol it is customary to obtain from the sp. gr., which should not exceed 0.799 at 15°/15° C., corresponding to 99.4 % methyl alcohol by volume (Table, p. 89). For a methyl alcohol sufficiently free from impurities to pass these tests, the sp. gr. method is usually satisfactory. For the determination of very small amounts of water in absolute methyl alcohol, the work of Gyr (*Ber.*, 1908, 41, 4322) on the esterification constant of phenyl acetic acid in methyl alcohol, and of Andrews (*J. Am. Chem. Soc.*, 1908, 30, 353) in applying Crimer's critical temperature of solution of alcohol with kerosene (Crismer, *Les températures critiques de dissolu-*

tion, Bruxelles, 1904) to the examination of absolute alcohol are especially significant, though no general use has been made of either.

In refining to produce reagent grade methyl alcohol the following tests are the most difficult to meet, hence are of special importance in judging a methyl alcohol reputed to be of high quality.

(a) *Odour*.—As the last traces of the impurities in the alcohol are removed there is a very marked improvement in odour; the last faint traces of the “rawness,” associated with the ordinary product, disappear and the alcohol acquires the “clean” odour, characteristic of pure ethyl alcohol but noticeably softer.

(b) *Empyreumatic Substances*.—When 5 c.c. of concentrated sulphuric acid are added to 5 c.c. of the alcohol, with cooling and shaking, the mixture should not show more than a faint yellow colouration. With exceptionally well-refined methyl alcohol, no colour at all is produced in this test.

(c) *Permanganate Test*.—When the permanganate test, carried out as described for ethyl alcohol (p. 135) is applied, the “permanganate time” should be not less than 10 minutes. With the best of these methyl alcohols the colour persists 30–40 minutes, and with exceptional samples, even 1 or 2 hours. As to detecting acetone, this test has, of course, no significance.

(d) *Acetone and Ethyl Alcohol*.—This test is usually made by mixing 5 c.c. of the alcohol with 50 c.c. of 2.0 N sodium hydroxide solution and adding, with repeated shaking, 25 c.c. of 2.0 N iodine solution. No turbidity or precipitate should appear, and after warming to 60–70° for half an hour no positive odour of iodoform should be perceptible. Here, as in the ether modification below, it is advisable to carry out at the same time a blank omitting only the addition of the sample. The test will detect about 0.015% of acetone and 1.0% of ethyl alcohol.

A more sensitive test is obtained by replacing the 2.0 N iodine solution with an equal volume of N/10 solution, and proceeding as above with certain precautions. The alkali solution used should be entirely clear. The advisability of using sodium hydroxide of the grade “C. P. by alcohol” in this connection has been questioned, but it seems to give entirely satisfactory results. On the other hand such samples of fused and electrolytic sodium hydroxide as have been tried, give perfectly clear solutions with much more difficulty. After mixing the test portion with the reagents, air bubbles should

be removed from the solution by applying an air pump; and the reacting solution should not be placed in bright light. After the turbidity and odour have been observed, the mixture is shaken up with 5 c.c. of alcohol-free ether. The presence of 0.1% ethyl alcohol, or about 0.001–0.002% acetone can be detected by the yellow colouration assumed by the ether layer which separates.

## ACETONE

*Dimethyl Ketone:*  $\text{CH}_3\text{CO.CH}_3$ .

Acetone is a colourless, pleasant-smelling, neutral liquid, miscible in all proportions with water, methyl alcohol, ethyl alcohol, ether, fatty acid esters, and chloroform. It can be separated from aqueous solution by adding calcium chloride. If its solution in water or dilute alcohol is saturated with calcium chloride and then shaken out with petroleum ether, the acetone is removed but the separation is not complete. Common salt, sodium sulphate, and potassium carbonate act in the same way. Acetone gives a crystalline compound with sodium iodide ( $\text{NaI} \cdot 3 \text{C}_3\text{H}_6\text{O}$ ) use of which has been suggested as an easy way of obtaining pure acetone (Shipsey and Werner, *Trans. Chem. Soc.*, 1913, 103, 1255). The crystalline addition product with sodium bisulphite has been used generally for this purpose.

**Physical Properties.**—The b. p. of acetone according to Fuchs (*Z. angew. Chem.*, 1898, 38, 870) ranges from 55.06 at 710 mm. to 78.16 at 790 mm. Young, in his recent book (*Distillation Processes and Principles*, 1922, 257) gives the b. p. as 56.2 to 56.3° at 760 mm. with an approximate change of 0.03° for each millimetre change in pressure. The m. p. is –94.9° according to Ladenburg and Krugel (*Ber.*, 1899, 32, 1821). The sp. gr. at 15°/4° according to MacElroy and Krug (*J. Anal. Chem.*, 6, 187) is 0.79726. These authors give a table showing sp. gr. at 15°/4°, of aqueous solutions of acetone and the table has been reprinted in *Chem. Centr.*, 1892, 2, 158, and in *J. Chem. Soc.*, 1893, 64, i, 7. Squibb (*J. Am. Chem. Soc.*, 1895, 17, 199) obtained sp. gr. 0.80816 at 4°/4°; 0.79662 at 15°/15°, 0.78699 at 25°/25° on an acetone, which from its method of preparation was unlikely to contain methyl or ethyl alcohol, but which probably was not free from water. Recently reported values given by Young are: Reilly and Ralph (*Sci. Proc. Royal Dublin Soc.*, 1919, 15, 598),

0.79123 at  $20^{\circ}/4^{\circ}$ ; Price (*Trans. Chem. Soc.*, 1919, **115**, 1125), 0.79082 at  $20^{\circ}/4^{\circ}$ ; Bramley (*Trans. Chem. Soc.*, 1916, **109**, 455), 0.7912 at  $20^{\circ}/4^{\circ}$ . For estimating by sp. gr. the amount of water in an otherwise pure acetone the curve plotted from the data given by Young (p. 261) is preferable to the older specific gravity tables.

**Detection of Acetone.**—For the detection of acetone, especially in urine, a great many tests have been described. Only four will be referred to here.

**Lieben's Iodoform Test** (*Z. Anal. Chem.*, 1870, **9**, 265).—Acetone gives the iodoform reaction in the cold, and in the probable absence of other impurities such as aldehyde and isopropyl alcohol which behave similarly, the formation of iodoform in the cold is useful as a test for acetone. To 2 c.c. of the liquid 3 to 5 drops of 10% sodium hydroxide are added and then, drop by drop, N/2 iodine solution until very faintly yellow. In the presence of acetone, iodoform separates at once. In water solution, as little as 0.005% acetone gives an immediate turbidity, and on long standing a smaller amount may be detected.

**Legal's Nitroprusside Test** (*J. Pharm. Chem.*, 1888, **17**, 206).—To 5 c.c. of the liquid 5 drops of a sodium nitroprusside solution (freshly prepared, or else made permanent by protecting from light) are added, and then 1 c.c. of 10% sodium hydroxide solution. In the presence of acetone the liquid assumes an orange tint, which fades to clear yellow in 15 to 20 minutes. If the experiment be repeated and the solution made just distinctly acid with acetic acid immediately after the addition of the alkali, a purplish-red colour will develop in the presence of acetone, and this colour remains practically unchanged for 15 to 20 minutes. The comparative persistence of this purple colour (it slowly changes becoming more blue) serves to distinguish acetone from higher homologues and from certain other substances which may occur in urine. Applied to dilute solutions of acetone in ethyl alcohol, this test will detect 0.05 to 0.10%. With the same solutions Hasse's modification (*Pharm. Centralb.*, 1920, **61**, 613) of Rothera's nitroprusside test (*J. Physiol.*, 1908, **37**, 491; *C. A.*, 1910, **4**, 2159) proved slightly more sensitive, *i. e.*, capable of detecting 0.01 to 0.02% of acetone. The latter is made by treating 0.5 c.c. of the sample, not over 50% alcohol, with 1 c.c. "ammonia mixture" and 0.05 to 0.10 c.c. of a 25% sodium nitroprusside solution. A permanganate like colour appears with

$\frac{1}{2}$  hour in case acetone is present. The "ammonia mixture" is made by dissolving 30 grm. ammonium sulphate in 45 c.c. hot water, cooling and adding 10 c.c. of 30% ammonia water. For small amounts of acetone the reaction mixture must be kept cool and at a fixed temperature. According to Jones (*Am. J. Pharm.*, 1919, 91, 732) acetone may be detected in ether by using the Rothera nitroprusside test as described by Wade and Finnemore. (Wade and Finnemore, *Pharm. Journ.*, 1914, 139 and 160.)

**Frommer's Salicylaldehyde Test.**—(*Berl. klin. Wochensch.*, 42, 1008, 1405.) This delicate test is made by adding, to 10 c.c. of the liquid to be examined, 1 grm. of solid potassium hydroxide and then without waiting for this to dissolve, 10 drops of salicylaldehyde, and warming to 70°. In the presence of acetone a purple-red contact ring develops. If the hydroxide is all dissolved before the addition of the salicylaldehyde, the liquid becomes yellow, then reddish and finally purple-red. According to Kolthoff (*Pharm. Weekblad.*, 1918, 55, 1021; *C. A.*, 1918, 12, 2180), it is possible to detect by this reaction as little as 0.0001% acetone in water solution. He states also that vanillin is equally sensitive and has the advantage of being specific for acetone—neither methyl-ethyl ketone nor aromatic ketone giving the reaction.

**Reynolds-Gunning Mercuric Oxide Test** (*Z. anal. Chem.*, 1885, 24, 148).—This test was originally made by adding the liquid suspected of containing acetone, to mercuric chloride made strongly alkaline with alcoholic potassium hydroxide, shaking vigorously, and testing the perfect clear filtrate for mercury by means of ammonium sulphide, or by stannous chloride in acid solution.

Applied to the detection of acetone in reagent grade ethyl alcohol, the test is carried out as follows: shake for 1 minute a mixture of 6 c.c. of barium hydroxide water (3.3%), 6 drops of mercuric chloride solution (1:20) and 2 c.c. of the alcohol to be tested. The clear filtrate must not show a dark colour upon the addition of ammonium sulphide. The test will detect 0.02% acetone. (Merck, *Chemical Reagents. Their Purity and Tests*, 1914, 33; Murray, *Standards and Tests for Chemical Reagents*, 1920, 72).

**Detection of Acetone in Urine.**—In the previous edition attention was directed to the series of papers by Bohrisch (*Pharm. Centralb.*, 1907, 48, 181, 206, 220, and 245) giving a review of the many methods which had been described up to that time. The

number has steadily increased since and the methods extended to blood, milk, and other physiological liquids. The interest of the general analyst however does not seem to warrant a more extended treatment than was given formerly. The following tests are simple and widely used. If care is taken to judge by more than one method, they are usually sufficient.

The salicylaldehyde test may be applied to the urine directly and it has the advantage that acetoacetic acid, which may be present, does not give the reaction. If no red or reddish ring develops, but only a yellow colouration, acetone is certainly absent. On the other hand the reaction is so sensitive that it gives no idea of the quantity present. If a positive result is obtained, therefore, a less delicate test should be applied, preferably the nitroprusside test, which may also be applied to the urine directly without distillation or other previous treatment. A positive result indicates a notable quantity of either acetone or acetoacetic acid. To distinguish between these, many methods have been described. Bohrisch recommends acidifying 50 c.c. of the urine with sulphuric acid and shaking with 25 c.c. of ether. The ether is then shaken with 15 to 20 c.c. of water, which will then contain a large part of the acetone and acetoacetic acid originally present, while other substances which interfere with the tests subsequently to be applied are removed. The aqueous layer is freed from dissolved ether by warming to 40° with frequent shaking, and a portion is then tested for acetoacetic acid by means of ferric chloride. If no violet colouration results, then the positive reaction with nitroprusside must have been due to acetone. But if acetoacetic acid is shown to be present, the remainder of the ether-free aqueous extract is tested for acetone by the iodoform test. This test should not be applied to urine directly, because other substances which give the reaction may be present, and the above described method, though far from quantitative, is preferable to any distillation method because at the temperature of distillation acetoacetic acid and other substances may be decomposed yielding acetone.

**Estimation of Acetone.**—Messinger's volumetric method (described under "Assay of Wood Spirit") is the one most frequently used. Devised originally to replace Kramer's gravimetric iodoform method (*Ber.*, 1880, 13, 1000) for testing methyl alcohol for acetone, this method has been in use in a great variety of cases, and for all degrees of acetone concentration. Care must be taken to remove, or

to correct for other substances, such as acetaldehyde, isopropyl alcohol, and to a slight extent ethyl alcohol, which also gives iodoform under the conditions of this test. One marked advantage the Messinger test has over others is that, due to wide usage, its limitations have come to be much better known.

Much contradictory information has been published on details for carrying out the test. A considerable part of the difficulty seems to have been due to failure to take into account the volatility of acetone, and to irregularities inherent in the iodometric titration rather than to imperfect conversion of the acetone to iodoform. When employed with care the method gives very consistent results. Goodwin, (*J. Am. Chem. Soc.*, 1920, **42**, 39) recently reported satisfactory results on high percentage acetones, and Marriott (*J. Biol. Chem.*, 1913, **16**, 281) found it satisfactory when applied to dilute (0.1%) solutions. The procedure recommended by Goodwin and given in this chapter under "Assays of Wood Spirit" is essentially the same, except for strengths of solutions used, as the British Government Laboratory methods.

**Denigès' and Other Mercury Reagent Methods.**—Several quantitative methods, based on the formation of insoluble mercury complexes with acetone, have been proposed. That of Denigès (*Compt. rend.*, 1898, **127**, 693; *Bull. soc. chem.*, 1899, (v), **19**, 754) depends on the formation of an insoluble compound when acetone is treated with a large excess of mercuric sulphate. The reagent is prepared by dissolving 5 grm. of mercuric oxide in 100 c.c. of water to which 20 c.c. of sulphuric acid have been added. The liquid under examination is, if necessary, diluted with water until its content of acetone is reduced to 0.2%; the content of methyl alcohol also must not exceed 50%, nor that of ethyl alcohol 2%. To 25 c.c. of the diluted liquid 25 c.c. of the mercury reagent is added, and the whole heated on the water bath for 10 minutes. After cooling, the precipitate is collected on a tared filter, washed with not more than 100 c.c. of cold water; dried at 100°, and weighed as  $3\text{Hg}_5\text{S}_2\text{O}_{11} \cdot 4\text{C}_3\text{H}_6\text{O}$ . The weight of the precipitate multiplied by 0.0609 gives the weight of the acetone in the 25 c.c. of liquid taken for the experiment. Denigès' proposed a volumetric modification, using a mercury solution of exactly known strength and estimating the unused mercury in the filtrate, using standard potassium cyanide and silver nitrate solutions.

The Scott-Wilson method (*J. Physiol.*, 1911, **42**, 445) and modi-

fications proposed by Marriott (*Jour. Biol. Chem.*, 1913, 16, 281) and by Folin and Denis (*J. Biol. Chem.*, 1914, 18, 263) make use of the insoluble complex formed by an alkaline solution of basic mercuric cyanide. The reagent is prepared by allowing 400 c.c. of a 0.7268% solution of silver nitrate to flow slowly into a solution of 10 grm. of mercuric cyanide and 180 grm. of sodium hydroxide in 1200 c.c. water, the solution being well shaken during the addition. At least 30 c.c. of reagent must be taken per milligram of acetone and the test portion should not contain more than 5 mg. of the latter. The use to which this method is particularly suitable seems to be the examination of physiological fluids, such as milk, blood, normal urine, etc., the acetone content of which is too small to be dealt with satisfactorily by other methods. It is claimed that the reagent is not affected by alcohol, but a precipitate forms with very small amounts of aldehydes, chlorides, hydrogen sulphide, and ammonia. Therefore assurance as to the absence of these compounds is necessary before the results can be considered reliable. Another difficulty with the method is the uncertainty as to the composition of the precipitate. For this reason Marriott's method of obtaining the "acetone factor" of the sulphocyanate solution, by applying the procedure used to accurately known dilute solutions of acetone seems preferable to standardizing against an analyzed solution of mercuric nitrate. The method given by Marriott is as follows:

"Dilute solutions of pure acetone are run into an excess of the recently filtered reagent contained in small Erlenmeyer flasks, allowed to stand 20 minutes and then filtered through an asbestos mat in a separable-bottom Gooch crucible. By first filtering an aqueous suspension of talcum powder so as to partly close the pores of the filter, less difficulty is experienced in obtaining clear filtrates. In some cases the first portions of the filtrate are turbid and have to be refiltered. The precipitate is washed with cold water until the washings are free from silver.

"With the aid of a pointed hooked glass rod the precipitate, mat, and crucible bottom are transferred to a 50 c.c. beaker, any adhering particles of the precipitate being washed into the beaker with about 10 c.c. of 'acid mixture' (nitric acid 40 parts, sulphuric acid 5 parts, water 55 parts). 1 c.c. of N/5 potassium permanganate is added, the beaker covered with a watch glass, and the liquid boiled until colourless. More permanganate is then added a few drops at a time

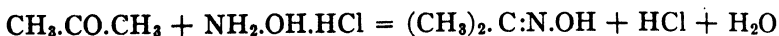
until a persistent brown colour is obtained which does not disappear on boiling for a couple of minutes. The brown colour is then discharged by the addition of a few drops of strong yellow nitric acid. The greater the amount of acetone present the more permanganate is required, and it is essential to the accuracy of the method that an excess be added as indicated above, otherwise the results are low.

"The beaker is cooled under the tap, 2 c.c. of saturated ferric alum added, and a standard solution of potassium sulphocyanate (approx. 0.1%) run in from a burette until a very faint pinkish brown colour is obtained throughout the solution. The end-point, which consists in the faintest trace of colour, can be detected only when the titration is performed on a pure white surface. A control beaker with 1 drop excess sulphocyanate should be at hand for comparison. A whole cubic centimeter of sulphocyanate may be run in after the end-point is reached without very greatly darkening the shade." As indicated above, the "acetone factor" of the sulphocyanate is obtained by analyzing in the same way a known dilute solution of pure acetone, standardized by weighing or by analysis using the Messinger method.

For estimating very small amounts of acetone (in blood) Marriott (*J. Biol. Chem.*, 1913, **16**, 289) obtained satisfactory results by measuring with a nephelometer the precipitate produced by the Scott-Wilson reagent. Folin and Denis (*J. Biol. Chem.*, 1914, **18**, 263) combined turbidity measurement with removal of acetone by aeration, thereby obtaining a simple method for estimating preformed acetone in urines containing only small amounts.

The Scott-Wilson method was applied by Jones (*Am. J. Pharm.*, 1919, **91**, 732) to the determination of acetone in ether.

**Hydroxylamine Hydrochloride Method.**—If acetone is allowed to react on a neutral solution of hydroxylamine hydrochloride, acetoxime and hydrochloric acid are formed. Titration of the liberated hydrochloric acid by a standard alkali, using methyl orange indicator, gives a measure of the acetone present. The reaction is quantitative and proceeds according to the equation:



Neither methyl nor ethyl alcohol interferes. Acetaldehyde reacts in the same way as acetone, and, if present in significant quantity as compared with the acetone, must either be removed or be deter-

mined separately. The fact that ethyl alcohol does not interfere makes the method particularly useful for estimating acetone in alcoholic products, that is, in those cases where the Messinger method is least satisfactory. The method is discussed more fully under the detection of denatured alcohol.

**Estimation of Acetone in Urine.**—Distillation and estimation of acetone in the distillate by Messinger's method (Messinger-Huppert. See Neubauer and Vogel, *Analyse des Harns*, 3rd ed., 1890, p. 471) is most usual. Acetoacetic acid if present will be decomposed at the temperature of distillation, yielding acetone. For their separate estimation, Folin's aeration method (*J. Biol. Chem.*, 1907, **3**, 177; 1912, **11**, 499; 1914, **18**, 263) is to be preferred. For methods applicable to urines of extremely low acetone content refer to the discussion of the Scott-Wilson method above. By combining chromic acid oxidation with distillation—the acetoacetic acid and pre-formed acetone having been first removed by boiling—oxybutyric acid may be determined through the acetone produced (Shaffer, *J. Biol. Chem.*, 1913, **16**, 265). In exacting cases the problem of interfering substances becomes very important and the original literature should be consulted.

**Analysis of Acetone.**—Most of the acetone of commerce is of good quality. The best commercial grade is approximately that of an acetone refined for use in making cordite. The British Government specifications require that for this purpose the product be genuine acetone, containing no other ingredient except small quantities of substances which are normal by-products of its manufacture. It must be colourless, absolutely transparent, and when mixed with distilled water in any proportion must show no turbidity. It must leave no residue when evaporated on a boiling water bath and its sp. gr. at 15.5°/15.5° must not exceed 0.800. The "permanganate test time" must not be less than 30 minutes when made as follows: 100 c.c. of the acetone is mixed with 1 c.c. of 0.1% potassium permanganate solution, kept at 15.5°, and the time elapsing between mixing and final disappearance of the permanganate colour is observed. It is required further that the acetone contain not more than 0.002% of carbon dioxide and be otherwise quite neutral.

According to Marshall (*J. Soc. Chem. Ind.*, 1904, **23**, 646) deterioration on long storage (fall in permanganate time and darkening in colour) may be caused by the presence of a small amount

either of basic substances, or of strong acid. For estimating these a suitable portion is diluted with an equal volume of water, 2 to 4 drops of a saturated water solution of paranitrophenol added, and the mixture titrated using standard acid or alkali as the case may require. Weak acids may be determined by mixing another portion with water as before, boiling for 5 or 10 minutes and titrating with standard alkali after the addition of phenolphthalein indicator. The difference between this titration and one similarly made except that boiling is omitted, gives a measure of the carbon dioxide present.

In case an actual determination of acetone is required, the Messinger method as described under the assay of wood spirit is used. To avoid loss of volatilization the proper amount of sample (about 0.5 grm.) is weighed out in a thin-walled sealed glass bulb, which is then introduced into a 500 c.c. graduated flask, three-fourths full of water. The bulk is broken under the surface of the water by means of a flattened glass rod, and the solution made to volume at the desired temperature. To withdraw aliquots (25 c.c.) it is advisable to force the solution up into the pipette, rather than draw it up, and to deliver the solution under the surface of the liquid in the titration flask. A source of error not found recorded in the literature lies in the fact that an acetone to which a trace of strong acid (sulphuric) has been added, may acquire the power of using up much more than the normal amount of iodine. In one observed instance a 99.5% acetone, to which 0.05% of strong sulphuric acid had been added, showed 130% "acetone" by the Messinger method after 8 weeks standing.

Refined or reagent grade acetone is prepared from a commercial product, such as that above, by further purification. This may consist of liberation from the bisulphite or similar compound, of chemical removal of the impurities with subsequent rectification, or even of careful rectification alone. The product should meet easily all the tests given above. In addition it should show greater stability and higher permanganate time. Well refined acetones, examined by the permanganate test as recommended for refined alcohol, usually give permanganate times of 2 hours or more, sometimes much longer. The alcohol test is more sensitive than that for cordite acetone (only  $\frac{4}{5}$  as much permanganate being used) and is preferred in alcohol testing laboratories because it avoids

confusion. Furthermore, the refined acetone should be free from the foreign odours associated with commercial acetone. For specially denaturing cologne spirits to be used in compounding perfumes and lotions, the question of odour is important. Both the original odour and that after dilution with twice its volume of water are observed. The acetone is then mixed with a standard alcohol in the denaturing proportions, and odours again observed, diluted and undiluted, as before. As controls, samples found by experience to be acceptable are examined in this way at the same time. Such an acetone should show at least 99% by the Messinger method, the remaining 1% being practically all water. In case a determination of the water content in refined acetone is required, the only satisfactory method available is to determine sp. gr. at 20°/20° and refer to the table given by Young (*Distillation Processes and Principles*, p. 261).

**Acetone Oils.**—In examining samples of acetone less carefully fractionated, the following observations of Heikel (*Chem. Zeit.*, 1908, **32**, 75) are useful. The higher ketones react with iodine and with mercuric sulphate but consume less of the reagent per unit weight than does acetone. Thus the fraction known in the trade as “ketones” (mainly ethyl methyl ketone), sp. gr. 0.811 to 0.815, appears to contain 90% of acetone by Messinger’s method and 63.5% by that of Denigès. The “light acetone oil” of the trade, sp. gr. 0.82 to 0.83, appears to contain 57% of acetone by Messinger’s method and only 32.5% by that of Denigès. This fraction contains little or no acetone really, but it is the apparent acetone content by the two methods or the Denigès-Messinger ratio which is valuable in judging the sample. For acetone itself the ratio is of course 1, for “ketones,” as the above numbers show, about 0.7, and for light oil about 0.57. Moreover, the mercury precipitate with “ketones” is no longer white, but yellowish, while that from the light oils is yellowish-brown.

More recently, Jones (*J. Soc. Chem. Ind.*, 1919, **38**, 180T) has proposed to determine the approximate composition of typical oils from acetone refining by the following procedure: (1) Estimate the water by throwing it out of solution by benzene or toluene, and measuring the amount separated. (2) Dehydrate over sodium sulphate, and distil through an efficient fractionating column, the distillate being collected in 3 fractions, *viz.*, below 68°, 68° to 90°,

90° to 140°. (3) Then from each fraction extract the ketones by means of strong sodium bisulphite solution (1.2 to 1.3 sp. gr.) and measure the volumes of residual, non-reactive substance. On artificial mixtures prepared to represent light and heavy acetone oils, and first runnings, satisfactory returns were obtained, but no results from the application of the method to commercial products were reported.

Only the light acetone oils are of importance as nitrocellulose solvents. They are excellent solvents, the heavy oils on the other hand being very poor (Conley, *J. Ind. Eng. Chem.*, 1915, 7, 885).

**Diacetone Alcohol**  $[\text{CH}_3)_2\text{C.OH.CH}_2\text{.CO.CH}_3]$ .—This condensation product made by the action of alkalis on acetone came into notice during the later days of the war as a solvent for cellulose acetate in preparing “dopes” for coating aeroplane wings, and is finding some general application since as a minor constituent of preparations of that type. As obtained commercially it is a colourless to light straw-coloured liquid, with a mild and characteristic odour (sometimes said to resemble crushed green grapes). It is freely miscible with water and also with all the common solvents used in preparing nitro-cotton and cellulose acetate solutions. Distilled under atmospheric pressure, it boils roughly from 80 to 150°, with a good deal of decomposition. Traces of alkali tend to cause reversion to acetone. For this reason the product may be found to contain traces of free tartaric or some similar acid left in for protection. The content of diacetone alcohol is judged by the sp. gr., which for the commercial product is 0.900 to 0.910 at 15.5°/15.5°, corresponding to 80% or over of the alcohol. In reaction it should be neutral or acid to the extent of not more than 0.01% tartaric acid. No satisfactory method has been proposed for its estimation.

## ETHYL ALCOHOL

**Ethyl Alcohol, Methyl Carbinol**  $\text{C}_2\text{H}_5\text{OH}$ .—Pure ethyl alcohol is a colourless, nearly odourless, mobile liquid, possessed of a burning taste. Its b. p. varies from 76.36° at 710 mm. to 79.31° at 790 mm. (Fuchs, *Zeit. angew. Chem.*, 1898, 38, 870). It solidifies at  $-112.3^\circ$  (Ladenburg and Krugel, *Ber.*, 1899, 32, 1818). The sp. gr. is very close to 0.79390 at 60°/60° F. The value 0.79389 obtained by Osborne, McKelvy and Bearce (*Bull. Bureau of Standards*, 1913, 9,

327-474) is used as the basis of the 60°/60° F. alcohol table of the Bureau of Standards (*Circ. No. 19, "Standard Density and Volumetric Tables,"* 1916, 10-11). The agreement of this with some of the most frequently quoted earlier numbers is shown by the following, taken from McKelvey's recalculation to 25°/4° of the density determinations recorded between 1864 and 1913:

Observer	D. 25°/4°	Dehydrating agent	References
Mendeleeff.....	0.78504	CaO + BaO	<i>Z. für. Chem.</i> , 1865, 257 to 264. <i>Ann. Phys. Chem.</i> , 1869, 138, ii, 138, 103, 250.
Young.....	0.78507	Distillation with hexane	<i>J. Chem. Soc.</i> , 1902, 81, 707-17.
Klason-Norlin.....	0.78508	Ca	<i>Arkiv. Kem. Min. Geol.</i> , 1906, 2, 93.
Bureau of Standards..	0.78507	CaO, Al-Hg	( <i>loc. cit. Bull. 9</i> ).

Tables based on the Bureau of Standards data are now in general use throughout the United States. The tables most commonly in use in England are based on the earlier (1847-8) work of Fownes and Drinkwater, using an alcohol of a sp. gr. of 0.7938, while the tables of Tralles, whose alcohol had a sp. gr. of 0.7946 are still the basis of excise work.

The coefficient of expansion of alcohol is very large, a point of considerable importance to the analyst, who usually estimates alcohol from the sp. gr. of its aqueous solutions.

Alcohol is miscible with water in all proportions, a considerable evolution of heat and contraction in bulk taking place on admixture. The two form a minimum-boiling mixture, which at 760 mm. pressure contains 97.45% alcohol by volume (Noyes, *J. Am. Chem. Soc.*, 1901, 23, 467). This figure the product of the customary redistillation tends to approach. As to the effect of reduction of pressure, Wade and Merriman (*J. Chem. Soc.*, 1911, 99, 997-1011) state that, as the pressure is decreased, the proportion of water in the minimum-boiling mixture decreases according to a regular curve until at 70 mm. the alcohol distils over anhydrous. Vrevski (*J. Russ. Phys. Chem. Soc.*, 1910, 42, 1349-55) obtained the following as the average of 30 to 40 tests at each of the three points: 97.3% (vol.) at 74.79°; 97.8% at 54.81°; and 98.5% at 39.76°.

*Rectified spirit of wine* is the British name given to the concentrated alcohol as produced by ordinary distillation. The rectified spirit of the British Pharmacopœia is described as containing 84% by weight of real alcohol, and having a sp. gr. of 0.838.

*Proof spirit* of the British Pharmacopœia has a sp. gr. of 0.920 which corresponds to a strength of about 49% by weight of real alcohol. The term "proof spirit," is very confusing to many people, and might with advantage be abandoned. Of this there is little chance at present, as it is adopted in several Acts of Parliament, and is the scale to which Sykes' hydrometer, used by the Excise, has reference. The Excise formerly tested the strength of spirits by pouring a certain amount on gunpowder. A light was then applied. If the spirit was above a certain strength ("proof") the gunpowder ultimately inflamed, but if weaker the gunpowder was too much moistened by the water to be capable of explosion, and the sample was said to be "under proof." By Act of Parliament, proof spirit is now defined to be a liquid of such density that, at 51° F., 13 vols. shall weigh the same as 12 vols. of water at the same temperature. The "proof spirit" thus produced has a sp. gr. of 0.91984 at 60°/60° F., and contains, according to Fownes, 49.24% by weight of alcohol and 50.76% of water. Spirits weaker than the above are described by the Excise as being so many degrees, or so much % "under proof" (U.P.). Thus, by the term "spirit of 20% or 20°, under proof," is meant a liquid containing, at 60° F., 80 vols. of proof spirit and 20 of water. "Spirit of 50° U.P." contains equal volumes of proof spirit and water, while pure water is 100° under proof. On the other hand, spirituous liquids stronger than proof spirit are described according to the number of volumes of proof spirit 100 vols. would yield when suitably diluted with water. Thus, "spirit of 50° O.P." is alcohol of such strength that 100 vols. at 60° F., when diluted with water to 150 vols., would be proof spirit.\* Absolute alcohol accordingly is 75¼° O.P., and contains 175.25% of proof spirit, for 100 vols. when diluted with water would yield 175.25 vols. of spirit at "proof." The relationship of percentages of absolute alcohol to those of proof spirit are explained below.

NOTE.—Owing to the contraction which occurs on mixing alcohol with water, the volume of water which it would be necessary to add in this instance would be considerably more than 50 vols. Thus, a mixture of 100 vols. of absolute alcohol with 60 of water measures only 154 vols. instead of 160.

In the United States the usual method of expressing the strength of alcohol is in volume per cent., but a "proof spirit," different from that of the British Excise, is also recognized. American "proof spirit" is "that alcoholic liquor which contains one-half its volume of alcohol of a sp. gr. 0.7939 at 60° F." The sp. gr. of such a spirit is (according to the Bureau of Standard tables) 0.93426 at 60°/60° F. It contains 42.49% of absolute alcohol by weight and 50 parts absolute alcohol and 53.71 parts of water by volume. Absolute alcohol contains 200% of proof spirit according to the American system instead of 175.25% as in the English system. The alcohol table (Table 7, 1913 edition) of the Gauger's Manual, used by the U. S. Department of Internal Revenue, is based on the Bureau of Standards data already referred to.

The customary strengths of alcohol handled in American trade have changed in recent years. Formerly much of the alcohol sold was "high wines," that is, the alcohol, usually about 65% by volume, obtained by a single ordinary distillation of the fermented mash. This grade was sold chiefly for liquor blending and was almost invariably "high wines" from grain alcohol. The redistilled alcohol was usually sold at 94% strength (188 proof). At present most of the alcohol is marketed at 95% (190 proof), some being sold as 96% and an increasing quantity as absolute alcohol. The finest of the 95% and 96% alcohols are also known as cologne spirits.

The United States Pharmacopœia (IX) designates three forms of alcohol: 1 *Absolute Alcohol*: Not less than 99% by weight. Sp. gr. not above 0.797 at 15.56° nor above 0.789 at 25°.

2. *Alcohol*: Not less than 92.3% by weight or 94.9% by volume at 15.56°. Sp. gr. not above 0.816 at 15.56° nor above 0.810 at 25°.

3. *Diluted Alcohol*: 41 to 42% by weight or from 48.4 to 49.5% by volume at 15.56°. Sp. gr. 0.935 to 0.937 at 15.56°, and from 0.930 to 0.932 at 25°C. This is a strength prepared by the pharmacist by dilution of the strong alcohol, and is not produced as such.

**Examination of Commercial Alcohol.**—Ordinary alcohol is commonly assumed to consist only of water and alcohol. This, however, is frequently far from true, commercial alcohol often containing distinct traces of higher homologues (fusel oil), aldehydes, acetic and other acids, esters of fatty acids, unsaturated compounds, volatile oils and various fixed impurities both organic and inorganic.

Considerable quantities of these naturally occur in "high wines" but in the rectification to produce 95% alcohol the amounts are reduced, for the most part, to traces. For detecting these, the methods developed earlier in connection with potable spirits are used chiefly, even though they are not always satisfactory when applied to industrial alcohol.

In manufacture, the quality of alcohol is judged mainly by the odour and "permanganate time" tests. The odour test is remarkably sensitive but requires experience and the observations are of a kind difficult to record and compare. The "permanganate time" test comes nearer to indicating the chemical purity of an alcohol than any single chemical test. Alcohols may be roughly graded as follows, with the test applied as directed below:

Poor quality.....	less than 5 minutes.
Fair.....	5 to 15 minutes.
Good.....	15 to 30 minutes.
High grade (cologne spirits).....	30 minutes or over

Alcohol specially prepared for refined chemical trade may test 40 or 50 minutes or even higher. Alcohol which tests 30 minutes and gives the clean, characteristic odour of pure alcohol, when examined by the odour tests below, may be safely pronounced a well-rectified product. However, an alcohol may have a soft and agreeable odour, and be preferable for certain uses, without showing up specially well in the permanganate test, since the permanganate-consuming impurities, though usually, are not necessarily of marked odour (see p. 136).

The alcohol may be examined further by the following more specific tests as the case requires.

1. *Alcohol Content*.—This is obtained from the sp. gr. determined as indicated later.

2. *Acidity*.—A 100 c.c. portion is titrated with N/10 or N/20 alkali, using phenolphthalein indicator, the result being expressed as acetic acid. The acidity thus found seldom exceeds 3 parts per 100,000, and is due in part to carbon dioxide. The latter may be practically removed beforehand by a 30 minute aeration with a strong current of air freed from carbon dioxide. In case non-volatile acid is to be determined apart from acetic, the method given under potable spirits is suitable.

3. *Residue*.—100 c.c. are evaporated in a counterpoised glass evaporating dish and the residue, if appreciable, dried at  $100^{\circ}$  and weighed. Usually only a non-weighable stain is obtained. If more than this, it is probably due to container contamination, which may be traced down by a further chemical examination of the residue. To completely avoid contamination of this sort is impossible, practically, where large quantities are to be handled.

4. *Esters*.—In extreme cases, ethyl acetate, ethyl butyrate, or amyl acetate, may be detected by odour. If a determination is necessary the method under potable spirits is used and the result calculated to ethyl acetate. The presence of considerable ester is desired in rum. High wines contain amounts which vary according to source and treatment and which are reduced to traces (2 to 5 parts per 100,000) in the redistilled alcohol.

5. *Methyl Alcohol and Acetone*.—These are not present in a reliable redistilled alcohol from the usual sources. On account of their extensive use in denaturing, however, contamination sometimes occurs. Methods of detection and estimation are given under Methyl Alcohol and Acetone and also under Denatured Alcohol (See p. 140). Methyl alcohol has been found present in considerable quantity in such samples of by-product alcohol from the cellulose industries as the writer has seen, but alcohol of this kind is not found in American trade.

6. *Furfural*. (Method under Potable Spirits).—In properly redistilled spirits only faint traces or none at all are present (3 parts per 100,000 is unusual even in such products as rum.)

7. *Aldehyde*.—For detection of aldehyde, resinification with caustic alkalies, the various silver nitrate reduction tests, Nessler's reagent, and the permanganate tests all give useful indications. The U.S.P. (IX) test, observing the absence of more than a faint brownish tinge when 20 c.c. of the alcohol and 1 c.c. of N/10 silver nitrate are allowed to stand for 6 hours in diffused daylight, will not show the presence of much less than 0.1% of *pure* acetaldehyde in high-test alcohol. The ammoniacal silver nitrate tests are somewhat more sensitive, the resinification test less so.

The British Pharmacopœia directs that the silver test be made by the addition of 30 fluid grains (2 c.c.) of N/10 silver nitrate to 4 fluid ounces (120 c.c.) of the sample to be tested. After exposure to good light for 24 hours and decantation from the black precipitate,

no further reduction of silver should be obtained on repeating the treatment, showing reducing substances to be present to the extent of less than about 2.5 grains (0.6 grm.) per pint (450 c.c.).

For distinguishing between different aldehydes, use may be made of the Barbet-Jandrier method (Girard and Cunaissé, *l'Analyse des Alcools*, p. 129) depending on the colour reactions produced with various phenols.

For accurate determination in redistilled alcohol use the method given, under Potable Spirits.

8. *Fusel Oil*.—Traces remain in the best grade of spirits and no really satisfactory method for quantitative determination, is known. The Allen-Marquardt method (under Potable Spirits) is the best available but gives low results. As the chief objection to fusel oil, from the industrial point of view, is its odour, a spirit in which fusel oil cannot be detected by carefully made odour tests as described below, is suitable for most purposes.

The U.S.P. qualitative test made by allowing 25 c.c. to evaporate spontaneously to near dryness, adding a few drops of concentrated sulphuric acid to the residue and noting absence of red or brown colouration, is useful for detecting alcohols from which the fusel oil fractions have been imperfectly removed. The limit of sensitiveness of the U.S.P. test is at an addition of about 30 parts per 100,000 of average crude to pure alcohol. This, like the odour test, is based not on the detection of fusel oil itself but of impurities associated with it. The test is critically discussed under the French Sulphuric Acid Method (p. 254). The writer has confirmed Veley's observation that pure isobutyl alcohol gives no colouration, and in addition found that very pure amyl alcohol also gives none.

9. *Molasses Alcohol*.—The examination of many alcohols from molasses has shown that on underlaying the alcohol with concentrated sulphuric acid and observing after one hour, neither redistilled alcohol from molasses nor that from other sources gives a rose-red contact zone. High wines from molasses alcohol may give a reddish cloud and it is probable that this test, as well as the prejudice against molasses alcohol dates back to the time when much alcohol was sold as high wines. As most of this was used for liquor blending, the characteristic odour of molasses high wines was highly undesirable. When the high wines are carefully redistilled the odour of alcohols from different sources tends to become the same and it is the

writer's belief that for the highest quality alcohols, properly redistilled in the same way by the continuous process, it is impossible to distinguish molasses and grain alcohol by odour.

10. *Permanganate Time Test.*—A glass stoppered cylinder is thoroughly cleaned, rinsed with distilled water and then with the alcohol to be tested. Cleaning with strong hydrochloric acid to remove oxides of manganese is advisable if the cylinder has been used previously for this test. A 50 c.c. portion of the alcohol to be tested is introduced, the cylinder and contents cooled to approximately  $15^{\circ}$  and 2 c.c. of a fresh potassium permanganate solution containing 0.2 gm. per litre added by means of a pipette, the exact time being noted. The contents are mixed at once by inverting the stoppered cylinder, which is then placed out of bright light and kept at  $15^{\circ}$  to  $16^{\circ}$ , using a water bath if necessary. The number of minutes required for the complete disappearance of the pink color is the permanganate time of the alcohol.

The test is so sensitive that with high-quality alcohols certain precautions must be observed if consistent results are to be obtained:

(a) Only alcohols of approximately the same strength should be compared. A 50 minute 95% alcohol will, if diluted to 65% test about 25 minutes (50 c.c. of the diluted alcohol being used). Alcohols are tested in the strengths at which they are sold.

(b) The temperature must be controlled, especially with high grade alcohols. A 95% alcohol testing 30 minutes at  $25^{\circ}$  had a 50 minute permanganate time at  $15^{\circ}$  to  $16^{\circ}$ .

(c) The reaction is accelerated by light. An alcohol testing 9 minutes in bright sunlight showed 4 minutes when the cylinder was kept as far as possible in a dark box during the test. (b) and (c) are sufficiently well-taken care of by using as a bath an asbestos-jacketed gallon can with a board cover carrying a thermometer—the can being filled with water at  $15^{\circ}$  at start of test.

(d) The reduction products of  $\text{KMnO}_4$  catalyze the reaction, hence necessity of dissolving manganese dioxide deposits from used cylinders and of using fresh permanganate solution. Acids hasten the decomposition but no alcohol is acid enough so that this need be considered.

(e) Extreme care in sampling and handling a high-quality alcohol is necessary. Short contact with a cork stopper is usually sufficient to ruin a sample so far as permanganate test is concerned. If glass

stoppered containers are not available, cork stoppers covered with clean tin-foil may be used safely.

(f) If difficulty is experienced in judging the end-point, Barbet's device (Girard and Cunaissé, *l'Analyse des Alcools*, p. 44) of using as a blank, the salmon-coloured solution obtained by diluting to 50 c.c. with distilled water, a mixture of 5 c.c. of cobaltous chloride (50 grm. per litre) and 7 c.c. of uranium nitrate (40 grm. per litre) may be used; or a second test may be started shortly after the first, which then serves as a blank.

The following possible impurities, if present in the amounts indicated, were found to be sufficient to reduce to 25 minutes the permanganate time of a 50 minute alcohol:

Acetaldehyde.....	35 parts per 100,000
Acrolein.....	0.15 part per 100,000
Allyl alcohol.....	0.5 to 1.0 part per 100,000.
Crude fusel oil.....	45 parts per 100,000

10. *Odour*.—Following closely the odours which develop as the alcohol evaporates from a clean filter paper wetted with a few c.c. of it, is a rough test frequently used.

More satisfactory results are obtained by observing odours from a special test goblet (London dock tester), with a 65 c.c. egg-shaped cup about  $2\frac{1}{2}$  in. deep and narrowing gradually to about  $1\frac{1}{4}$  in. in diameter at the top. Most of the odours are brought out sharply by dilution, also by warming. After the odour of the original is noted, a portion is diluted with 2 vol. of odourless distilled water, and the odour observed both after agitation, and after standing undisturbed for some time. If desired, the effect of warming to  $50^{\circ}$  or so may then be noted.

The average judgment of several observers on the same known standards showed the following as to limits of sensitiveness (observations at about  $25^{\circ}$ ):

Acetaldehyde.....	5 to 10 parts per 100,000
Crude fusel oil.....	3 to 5 parts per 100,000
Acrolein.....	0.05 to 0.08 part per 100,000
Allyl alcohol.....	uncertain even at 100 parts

The more extended procedure used especially for fusel oil by R. B. Crowell of the Western Industries Co. is capable of giving still better results. In this the alcohol (95%) is distilled, undiluted, through a Hempel column until one half has passed over. Having thus

eliminated the "heads" the residue is diluted, as above, for odour comparison. A blank of the very highest purity alcohol is treated similarly, and also standards made from the pure alcohol, to which known amounts of the crude fusel oil under consideration have been added. Additions of from 1 to 5 parts per 100,000, depending on the type of fusel oil, can be detected without difficulty. The distillates, examined for odour in the same way, yield information as to the "heads" impurities (aldehydes, etc.).

For examining as to odour, alcohol to be used by the perfumer, Slack (*Perfumery and Essential Oil Record*, August, 1916, p. 268) recommends shaking 50 c.c. of water, 2 c.c. of the alcohol, and 1 c.c. of strong sulphuric acid in a thoroughly clean 8 oz. stoppered bottle and observing the odour by smelling directly from the bottle.

**Absolute Alcohol.**—With respect to all impurities except water, the tests given above for commercial alcohol are applicable to absolute.

The U. S. P. (IX) and Murray's Standards and Tests for Chemical Reagents fix the minimum alcohol content at 99.0% by weight (99.4% by volume). Special absolute, 99.8% by volume, is also produced in large quantities.

For *estimation of the alcohol content*, a very accurate sp. gr. determination is made and the tables used, with the precautions given under "Estimation of Alcohol." This is the best method where single determinations are required but in cases where frequent tests are necessary, where reliable weights and pycnometers are not available, or where reliable checking of the sp. gr. method is needed, Crismer's critical solution temperature method, using purified kerosene as described by Andrews (*J. Am. Chem. Soc.*, 1908, **30**, 353) and by the Bureau of Standards (*Bull. Bureau of Standards*, 1913, **9**, 344-48), is excellent.

**Detection of Water.**—For detecting the presence of water in absolute alcohol, the critical solution temperature method referred to above is probably the most satisfactory. Shaking with anhydrous cupric sulphate and looking for blue colour taken on by the salt will serve to distinguish absolute from 96% alcohol, but the test is not sensitive enough to be of use in judging absolute alcohol. Shaking with a crystal of potassium permanganate to develop a pink colour in case water is present is equally unsatisfactory. The result is too dependent on the size of permanganate particles added. For example shaking pure alcohol containing 1% water with a single crystal ( $1 \times 1 \times$

2 mm.) failed to give an appreciable pink colour, while a high-grade absolute shaken with a pinch of finely powdered potassium permanganate gave a colour as intense as a 99% alcohol treated in the same way. Not only is the amount of salt dissolved dependent on the fineness and on the amount of shaking, but reference to the "permanganate time" test discussed later will show that the depth and permanence of the pink colour is greatly dependent on the purity of the alcohol. The use of calcium carbide as proposed by Yvon (*J. pharm. chim.*, 1897, 7, 100) is more satisfactory than the preceding. On adding a pinch of the powder to absolute alcohol no bubbles of gas are liberated and the liquid remains transparent, whilst if water is present bubbles of acetylene are liberated and the liquid becomes milky from the formation of calcium hydroxide. By comparing with known solutions treated in the same way, the presence of 0.3 to 0.5% water can be easily detected; with 0.1 to 0.2% differences in gas evolution can be observed but the turbidity indication is uncertain. Henle (*Ber.*, 1920, 53, 719-722) reported that by the use of a xylene solution of partially decomposed aluminum ethylate the presence of water in ethyl alcohol could be detected. C. S. Piggot in a study (unpublished) of this reagent confirmed Henle's observations and found that the tedious preliminary heating to partially decompose the aluminum ethylate could be avoided and an equally satisfactory reagent obtained by using pure aluminum ethylate and refluxing for some time (30 minutes) after dissolving the solid. The test is made by adding to 20 c.c. of the alcohol in a *dry* tube or cylinder of clear glass, 1 c.c. of a 10% xylene solution of the ethylate and examining for the appearance of a turbidity. Alcohol containing 0.15% water gave distinct turbidity in 20 minutes; 0.08% a turbidity in 3 hours and 0.05% a perceptible but faint opalescence in 24 hours. A 5% reagent is slightly more sensitive but apparently slower than a 10%, higher strengths than 10% show the opposite behaviour.

**Denatured Alcohol.**—Alcohol, suitably denatured so as to be unfit for drinking purposes, is free from duty in most countries. Formerly in Great Britain it was necessary only to add to the spirit one-ninth of its volume of partially purified wood naphtha (see Methyl Alcohol section for both British and United States specifications). Since alcohol denatured in this way can be deprived of its offensive taste and odour without great difficulty, the British Board of Inland Revenue subsequently directed the further addition of  $\frac{3}{8}$  of 1% of

mineral naphtha. In view of representations which were made to the Board that British manufacturers were placed at a disadvantage as compared with their foreign competitors by the regulations with regard to the use of duty-free alcohol for industrial purposes, these regulations have been revised, with the result that a very large number of denaturants are now permitted in place of crude naphtha.

In the United States the use of specially denatured alcohol, authorized for specific manufacturing purposes, has increased rapidly. About 100 combinations involving nearly the same number of different materials as denaturants, are permitted at present and the number is constantly being added to. As a result, the examination of these alcohols presents an increasingly complex problem. Since however each interests a limited circle as compared with the completely denatured alcohols, only methods intended primarily for the latter will be given here.

For the determination of *benzene* in alcohol, the method of Hodel and Winterfeld (*Chem. Zeit.*, 1908, **32**, 313) or Wolff's modification (*ibid.*, 1910, **34**, 1193) was formerly used, the latter being applied also to the separation of *ether* along with the benzene. For determining *benzene*, Babington and Tingle recently (*J. Ind. Eng. Chem.*, 1919, **11**, 555) proposed the following more satisfactory method for dilute solutions (0.5–2.0% benzol), which has been adopted by the Inland Revenue Department of Canada: mix 100 c.c. of the alcohol with 200 c.c. of water, and distil at the rate of 1 c.c. per minute. Collect the first 20 c.c. in a graduated Eggertz tube, and add to it 15 c.c. of  $\frac{1}{6}$  saturated potassium bichromate solution and 2 c.c. of hydrochloric acid, sp. gr. 1.2. After standing (15 minutes) until an olive-green colour develops, exactly 10 c.c. of petroleum ether (well washed) are added, the mixture thoroughly shaken and allowed to settle. The volume of the upper layer, less 10 c.c. gives the percentage of benzene in the sample. In the absence of other compounds showing more or less the same behaviour (hydrocarbons, acetone) the method is satisfactory.

Detection of the substitution of completely denatured for tax-paid alcohol is a frequent problem. Since the oldest and most widely used formula requires denaturing with wood alcohol and pyridine, the *proved* presence of methyl alcohol in commercial ethyl is sufficient to condemn it. Failure to find methyl alcohol however does not prove the original free from completely denatured alcohol, since three

of the six formulas at present authorized in the United States require the addition of no wood alcohol and other tests (odour, residue, reaction, separation of hydrocarbons by salt solutions, the benzene and ether tests above) are necessary to complete the answer. Furthermore methyl alcohol tests alone are insufficient where accidental contamination by denatured alcohol (containing methyl) is involved. Here it is advisable to examine for the more easily detected *pyridine* and *acetone*. This may be done by Hasse's method (*Pharm. Zentralh.*, 1920, **61**, 613) in which 5 c.c. of the alcohol and 5 c.c. of water are acidified (litmus) with dilute sulphuric acid, and distilled. The first 5 c.c. of distillate is used for the detection of acetone (nitroprusside, salicylaldehyde, or mercuric oxide test; see under Acetone). The evaporation is carried nearly to dryness, the residue cooled, and made alkaline with sodium hydroxide solution, the odour of pyridine being distinct on deep inhaling if the original contained 0.01% of pyridine. The residue is distilled from a small flask, the first 1 c.c. over treated with 0.3 c.c. of a 2% solution of mercuric chloride, and allowed to stand. White needles or hair-like clusters of the addition product separate in a short time if the original contained 0.01%. With 20 hours standing the sensitiveness is stated to increase probably 10 times, making pyridine detectable at about 0.001% in the alcohol. For a quantitative result 100 c.c. of the alcohol is treated in the same way, the bases distilled over from the alkaline solution, and titrated with N/10 sulphuric acid using methyl-orange indicator.

Estimation of acetone may be made by the hydroxylamine method as described by Reif (*Zeit. Unters. Nahr. Genussm.*, 1921, **42**, 81). 50 to 100 c.c. of the alcohol, diluted to 50 % and treated with 10 c.c. of N/1 sulphuric acid to retain pyridine, are distilled through an efficient column. Sufficient distillate to recover all the acetone is collected in a cylinder set in ice water and connected up so as to avoid loss of acetone by evaporation. Aldehyde, if present by the fuchsin test, is removed by heating for one to one and one half hours on a water bath under a good reflux condenser, a measured fraction of the distillate with 10 c.c. of 3% hydrogen peroxide and 0.5 c.c. of N/1 alkali. The acetone is then distilled out and collected as before. A suitable measured fraction of the distillate is added to a solution of 0.5 gm. pure hydroxylamine hydrochloride in about 30 c.c. of water, the salt solution having been first neutralized exactly to methyl

orange by adding N/10 alkali. After standing for 45 minutes the liberated acid is titrated with N/10 alkali, an equal portion of the amine salt similarly neutralized serving as a blank. The titration is continued until there is no further tendency to turn acid on standing. Each c.c. of N/10 alkali used corresponds to 0.0058 grm. acetone.

Hoepner had previously (*Zeit. Unters. Nahr. Genussm.*, 1917, 34, 453) recommended combination of the chromic acid oxidation method with the hydroxylamine method, applied to the original and to the distillate after oxidation, as a suitable method for determining acetone, acetaldehyde and alcohol in mixture. The writer has found an abbreviated form of this method very satisfactory for determining both acetone and alcohol when present together. The combination should also be useful in examining mixtures of ethyl and isopropyl alcohols.

*Methylated finish* is a British preparation sold by those who are not licensed as vendors of methylated spirit. It is made by dissolving a gum resin in methylated spirit, and the British excise insists that the proportion present shall not be less than 3 oz. in the gallon.

**Detection of Alcohol.**—The mere detection of alcohol is seldom important and, as a rule, it can be estimated quickly and with considerable accuracy. Methods for its detection in ether, chloroform and some other liquids from which it cannot be easily separated by distillation are described in the sections relating to those substances.

Alcohol gives the iodoform reaction (see "Detection of Acetone"), but only on warming, preferably to about 60° for 1 minute. This reaction can often be applied to the detection of alcohol, although it is given by many other substances. Of these substances, some give the reaction in the cold, others only after prolonged warming. If an aqueous liquid, on being neutralized and once or twice fractionally distilled through some simple still-head, yields a distillate with sp. gr. notably less than 1, and which gives the iodoform reaction, but only on warming, the presence of alcohol may be suspected, and in many cases the presence of any of the other substances which might depress the sp. gr. and give the iodoform reaction is so improbable that such a distillate is practically a proof of the presence of alcohol. There is almost no limit to the sensitiveness of this test, if the number of distillations is increased. Kunz (*Zeit. anal. chem.*, 1920, 59, 302) is reported to have modified Lieben's reaction so as to make it a sensitive test for traces of ethyl alcohol. For identification, numer-

ous compounds have been suggested. Mulliken (*Identification of Org. Compounds*, I, p. 168) converted to ethyl 3.5 dinitrobenzoate m. p. 92 to 93, uncorrected. Recently, Reid (*J. Am. Chem. Soc.*, 1917, 39, 1249-55), proposed converting to ethyl nitrobenzyl phthalate m. p. 80°. Other examples may be found easily in the literature

E. Merck (*Chem. Zeit.*, 1896, 20, 228) proposed the following modification of Davy's test: pure molybdic acid is dissolved in warm strong sulphuric acid, and the resulting solution poured through the liquid under examination in a test-tube, both being kept as nearly as possible at a temperature of 60°. In presence of alcohol a blue ring appears at the junction between the two liquids, which is the more intense the larger the proportion of alcohol present. On shaking, the colour disappears, but by addition of a further quantity of the reagent it may be reproduced. The test is, of course, not characteristic of alcohol only, but it will detect even 0.02% of ethyl alcohol and 0.2% of methyl alcohol in aqueous solution. The writer has found this test to be as good for ether and iso-propyl alcohol as for ethyl alcohol; slightly less so for acetic ether, N-propyl and isobutyl alcohols, and still less sensitive for iso-amyl and N-butyl alcohols. For very pure methyl alcohol or for acetone, additions of 2-5% were required to give a distinct colouration.

Denigès (*Bull. soc. pharm. Bordeaux*, 1910, 50, 417; C.A., 1911, 5, 353) proposed a method for detecting ethyl in methyl alcohol based on the fact that by warming 0.2 c.c. of the alcohol and 5 c.c. of bromine water on a water bath till the bromine colour is nearly destroyed, appreciable amounts of aldehyde are shown by the fuchsine-bisulphite test when ethyl alcohol is present and none, or mere traces, when the methyl alcohol is pure.

**Estimation of Alcohol.**—Special methods for the estimation of ethyl alcohol in admixture with wood-spirit, chloroform, ether, etc., are described in the sections devoted to these substances. Ordinarily, the estimation of alcohol is effected by separating it from fixed substances by distillation, and then ascertaining the proportion of alcohol present in the spirituous liquid condensed. This is practically the *estimation of alcohol in mixtures consisting essentially of alcohol and water only*.

This is most generally effected by ascertaining the sp. gr. of the mixture. From the sp. gr., the percentage of absolute alcohol is readily found by referring to tables on the construction of which

great care has been bestowed by various observers, the subject being of great importance for excise purposes. For excise testing, a special glass or metal hydrometer is employed and the temperature carefully noted—elaborate, officially prescribed tables being used to ascertain the alcoholic strength. For laboratory testing, the sp. gr. bottle is a more satisfactory and accurate instrument. A bottle holding 50 or 100 c.c. is of suitable capacity for general use, but for some purposes a smaller one or a 10 c.c. Sprengel tube will be found serviceable. The manipulation is discussed in the "Introduction" (pp. 151) and some important special precautions are given below.

The proportion of alcohol contained in spirituous liquids may be expressed as: (1) percentage (of absolute) by weight, (2) percentage by volume, (3) percentage of proof spirit, or, (4) grm. of alcohol per 100 c.c.

Of these the first is probably the most satisfactory but custom requires more frequent use of others, the third being the usual British mode and the second the most-used method in the United States.

The following table gives the sp. gr. at 60°/60° F. and the per cent. of absolute alcohol by weight corresponding to various per cents. by volume, in tenths, from 0 to 100 vol. per cent. A table on the 60°/60° F. basis is chosen because the use of 60° F. as a reference temperature is very firmly rooted in industrial practice and commercial analyses usually must, in the end, be referred to that temperature. The more detailed table of the Association of Official and Agricultural Chemists (*A.O.A.C. Methods of Analysis*, 1920, 345–358) is for sp. gr. 20°/4° and is based on the same original data as the following.

The table given here is an abbreviation from the Standard Density and Volumetric Tables of the U. S. Bureau of Standards (*Circ.* No. 19, 1916). It was obtained as follows: the Bureau's Table 10 ("Relation between Weight per cent. and Unit Volume per cent.") was completed by calculating for the intermediate tenths of volume per cent., the corresponding weight per cent. (to third decimal). These results were combined with Table 3 (giving sp. gr. corresponding to every 0.1 vol. per cent., the sp. gr. being for air-saturated alcohol, weights in vacuo). The third decimals of the weight per cent. and the fifth of the sp. gr. figures were then dropped to the nearest figure of the next order, since a sp. gr. on alcohol accurate to the fifth place requires temperature control within about 0.01°, which is not usually attempted. Examination of absolute alcohol however,

Sp. Gr. at 60°/60° F. (15.56°/15.56° C.) of Mixtures of Alcohol and Water

Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight	Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight
100.0	0.79389	100.00	94.0	0.8198	91.02
99.9	0.79441	99.83	93.9	0.8202	90.89
99.8	0.79492	99.67	93.8	0.8206	90.75
99.7	0.79543	99.51	93.7	0.8210	90.61
99.6	0.79593	99.34	93.6	0.8213	90.47
99.5	0.79643	99.18	93.5	0.8217	90.33
99.4	0.79693	99.02	93.4	0.8221	90.20
99.3	0.79743	98.86	93.3	0.8224	90.06
99.2	0.79792	98.70	93.2	0.8228	89.93
99.1	0.79841	98.54	93.1	0.8231	89.79
99.0	0.79889	98.38	93.0	0.8235	89.65
98.9	0.7994	98.22	92.9	0.8239	89.52
98.8	0.7998	98.06	92.8	0.8242	89.38
98.7	0.8003	97.91	92.7	0.8246	89.25
98.6	0.8008	97.75	92.6	0.8249	89.11
98.5	0.8013	97.59	92.5	0.8253	88.98
98.4	0.8017	97.44	92.4	0.8256	88.85
98.3	0.8022	97.28	92.3	0.8260	88.71
98.2	0.8026	97.13	92.2	0.8263	88.58
98.1	0.8031	96.97	92.1	0.8267	88.44
98.0	0.8036	96.82	92.0	0.8270	88.31
97.9	0.8040	96.67	91.9	0.8274	88.18
97.8	0.8044	96.52	91.8	0.8278	88.04
97.7	0.8049	96.36	91.7	0.8281	87.91
97.6	0.8053	96.21	91.6	0.8284	87.78
97.5	0.8058	96.06	91.5	0.8288	87.65
97.4	0.8062	95.91	91.4	0.8291	87.52
97.3	0.8066	95.76	91.3	0.8295	87.38
97.2	0.8071	95.61	91.2	0.8298	87.25
97.1	0.8075	95.46	91.1	0.8301	87.12
97.0	0.8079	95.32	91.0	0.8305	86.99
96.9	0.8083	95.17	90.9	0.8308	86.86
96.8	0.8088	95.02	90.8	0.8312	86.73
96.7	0.8092	94.87	90.7	0.8315	86.60
96.6	0.8096	94.73	90.6	0.8318	86.47
96.5	0.8100	94.58	90.5	0.8322	86.34
96.4	0.8104	94.43	90.4	0.8325	86.21
96.3	0.8108	94.29	90.3	0.8328	86.08
96.2	0.8112	94.14	90.2	0.8331	85.95
96.1	0.8116	94.00	90.1	0.8335	85.82
96.0	0.8121	93.85	90.0	0.8338	85.69
95.9	0.8125	93.71	89.9	0.8341	85.56
95.8	0.8129	93.56	89.8	0.8345	85.43
95.7	0.8133	93.42	89.7	0.8348	85.30
95.6	0.8137	93.28	89.6	0.8351	85.18
95.5	0.8141	93.13	89.5	0.8354	85.05
95.4	0.8145	92.99	89.4	0.8358	84.92
95.3	0.8149	92.85	89.3	0.8361	84.79
95.2	0.8152	92.71	89.2	0.8364	84.66
95.1	0.8156	92.56	89.1	0.8367	84.54
95.0	0.8160	92.42	89.0	0.8371	84.41
94.9	0.8164	92.28	88.9	0.8374	84.28
94.8	0.8168	92.14	88.8	0.8377	84.15
94.7	0.8172	92.00	88.7	0.8380	84.03
94.6	0.8176	91.86	88.6	0.8383	83.90
94.5	0.8180	91.72	88.5	0.8387	83.77
94.4	0.8183	91.58	88.4	0.8390	83.65
94.3	0.8187	91.44	88.3	0.8393	83.52
94.2	0.8191	91.30	88.2	0.8396	83.40
94.1	0.8195	91.16	88.1	0.8399	83.27

Sp. Gr. at 60°/60° F. (15.56°/15.56° C.) of Mixtures of Alcohol and Water—(Continued)

Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight	Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight
88.0	0.8402	83.14	82.0	0.8582	75.86
87.9	0.8406	83.02	81.9	0.8585	75.74
87.8	0.8400	82.89	81.8	0.8587	75.62
87.7	0.8412	82.77	81.7	0.8590	75.51
87.6	0.8415	82.64	81.6	0.8593	75.39
87.5	0.8418	82.51	81.5	0.8596	75.27
87.4	0.8421	82.39	81.4	0.8599	75.15
87.3	0.8424	82.27	81.3	0.8601	75.04
87.2	0.8427	82.15	81.2	0.8604	74.92
87.1	0.8430	82.02	81.1	0.8607	74.80
87.0	0.8434	81.90	81.0	0.8610	74.69
86.9	0.8437	81.77	80.9	0.8613	74.57
86.8	0.8440	81.65	80.8	0.8616	74.45
86.7	0.8443	81.53	80.7	0.8618	74.34
86.6	0.8446	81.40	80.6	0.8621	74.22
86.5	0.8449	81.28	80.5	0.8624	74.10
86.4	0.8452	81.15	80.4	0.8627	73.99
86.3	0.8457	81.03	80.3	0.8630	73.87
86.2	0.8458	80.91	80.2	0.8632	73.76
86.1	0.8461	80.79	80.1	0.8635	73.64
86.0	0.8464	80.66	80.0	0.8638	73.53
85.9	0.8467	80.54	79.9	0.8641	73.41
85.8	0.8470	80.42	79.8	0.8643	73.29
85.7	0.8473	80.29	79.7	0.8646	73.18
85.6	0.8476	80.17	79.6	0.8649	73.06
85.5	0.8479	80.05	79.5	0.8652	72.95
85.4	0.8482	79.93	79.4	0.8655	72.83
85.3	0.8485	79.81	79.3	0.8657	72.72
85.2	0.8489	79.68	79.2	0.8660	72.60
85.1	0.8491	79.56	79.1	0.8663	72.49
85.0	0.8494	79.44	79.0	0.8666	72.38
84.9	0.8497	79.32	78.9	0.8668	72.26
84.8	0.8500	79.20	78.8	0.8671	72.15
84.7	0.8503	79.08	78.7	0.8674	72.03
84.6	0.8506	78.96	78.6	0.8677	71.92
84.5	0.8509	78.84	78.5	0.8679	71.80
84.4	0.8512	78.72	78.4	0.8682	71.69
84.3	0.8515	78.59	78.3	0.8685	71.58
84.2	0.8518	78.47	78.2	0.8687	71.46
84.1	0.8521	78.35	78.1	0.8690	71.35
84.0	0.8524	78.23	78.0	0.8693	71.23
83.9	0.8527	78.11	77.9	0.8696	71.12
83.8	0.8530	77.99	77.8	0.8698	71.01
83.7	0.8533	77.87	77.7	0.8701	70.89
83.6	0.8536	77.75	77.6	0.8704	70.78
83.5	0.8539	77.64	77.5	0.8706	70.67
83.4	0.8541	77.52	77.4	0.8709	70.55
83.3	0.8544	77.40	77.3	0.8712	70.44
83.2	0.8547	77.28	77.2	0.8714	70.33
83.1	0.8550	77.16	77.1	0.8717	70.22
83.0	0.8553	77.04	77.0	0.8720	70.10
82.9	0.8556	76.92	76.9	0.8723	69.99
82.8	0.8559	76.80	76.8	0.8725	69.88
82.7	0.8562	76.68	76.7	0.8728	69.77
82.6	0.8565	76.57	76.6	0.8731	69.65
82.5	0.8567	76.45	76.5	0.8733	69.54
82.4	0.8570	76.33	76.4	0.8736	69.43
82.3	0.8573	76.21	76.3	0.8739	69.32
82.2	0.8576	76.09	76.2	0.8741	69.21
82.1	0.8579	75.98	76.1	0.8744	69.09

Sp. Gr. at 60°/60° F. (15.56°/15.56° C.) of Mixtures of Alcohol and Water—(Continued)

Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight	Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight
76.0	0.8746	68.98	70.0	0.8900	62.44
75.9	0.8749	68.87	69.9	0.8902	62.33
75.8	0.8752	68.76	69.8	0.8905	62.23
75.7	0.8754	68.65	69.7	0.8907	62.12
75.6	0.8757	68.54	69.6	0.8910	62.02
75.5	0.8760	68.43	69.5	0.8912	61.91
75.4	0.8762	68.31	69.4	0.8915	61.80
75.3	0.8765	68.20	69.3	0.8917	61.70
75.2	0.8768	68.09	69.2	0.8920	61.59
75.1	0.8770	67.98	69.1	0.8922	61.49
75.0	0.8773	67.87	69.0	0.8924	61.38
74.9	0.8775	67.76	68.9	0.8927	61.27
74.8	0.8778	67.65	68.8	0.8929	61.17
74.7	0.8781	67.54	68.7	0.8932	61.06
74.6	0.8783	67.43	68.6	0.8934	60.96
74.5	0.8786	67.32	68.5	0.8937	60.85
74.4	0.8788	67.21	68.4	0.8939	60.75
74.3	0.8791	67.10	68.3	0.8942	60.64
74.2	0.8793	66.99	68.2	0.8944	60.54
74.1	0.8796	66.88	68.1	0.8946	60.43
74.0	0.8799	66.77	68.0	0.8949	60.33
73.9	0.8801	66.66	67.9	0.8951	60.22
73.8	0.8804	66.55	67.8	0.8954	60.12
73.7	0.8806	66.44	67.7	0.8956	60.01
73.6	0.8809	66.33	67.6	0.8958	59.91
73.5	0.8812	66.22	67.5	0.8961	59.80
73.4	0.8814	66.11	67.4	0.8963	59.70
73.3	0.8817	66.00	67.3	0.8966	59.59
73.2	0.8819	65.89	67.2	0.8968	59.49
73.1	0.8822	65.78	67.1	0.8970	59.38
73.0	0.8824	65.67	67.0	0.8973	59.28
72.9	0.8827	65.57	66.9	0.8975	59.17
72.8	0.8830	65.46	66.8	0.8978	59.07
72.7	0.8832	65.35	66.7	0.8980	58.97
72.6	0.8835	65.24	66.6	0.8982	58.86
72.5	0.8837	65.13	66.5	0.8985	58.76
72.4	0.8840	65.02	66.4	0.8987	58.65
72.3	0.8842	64.91	66.3	0.8990	58.55
72.2	0.8845	64.81	66.2	0.8992	58.45
72.1	0.8847	64.70	66.1	0.8994	58.34
72.0	0.8850	64.59	66.0	0.8997	58.24
71.9	0.8852	64.48	65.9	0.8999	58.14
71.8	0.8855	64.37	65.8	0.9001	58.03
71.7	0.8857	64.26	65.7	0.9004	57.93
71.6	0.8860	64.16	65.6	0.9006	57.83
71.5	0.8862	64.05	65.5	0.9008	57.72
71.4	0.8865	63.94	65.4	0.9011	57.62
71.3	0.8867	63.83	65.3	0.9013	57.52
71.2	0.8870	63.73	65.2	0.9015	57.41
71.1	0.8872	63.62	65.1	0.9018	57.31
71.0	0.8875	63.51	65.0	0.9020	57.21
70.9	0.8878	63.40	64.9	0.9022	57.11
70.8	0.8880	63.30	64.8	0.9025	57.00
70.7	0.8882	63.19	64.7	0.9027	56.90
70.6	0.8885	63.08	64.6	0.9029	56.80
70.5	0.8887	62.98	64.5	0.9032	56.70
70.4	0.8890	62.87	64.4	0.9034	56.59
70.3	0.8892	62.76	64.3	0.9036	56.49
70.2	0.8895	62.65	64.2	0.9039	56.39
70.1	0.8897	62.55	64.1	0.9041	56.29

Sp. Gr. at 60°/60° F. (15.56°/15.56° C.) of Mixtures of Alcohol and Water—  
(Continued)

Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight	Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight
64.0	0.9043	56.18	58.0	0.9178	50.17
63.9	0.9046	56.08	57.9	0.9181	50.07
63.8	0.9048	55.98	57.8	0.9183	49.97
63.7	0.9050	55.88	57.7	0.9185	49.87
63.6	0.9053	55.78	57.6	0.9187	49.77
63.5	0.9055	55.67	57.5	0.9189	49.68
63.4	0.9057	55.57	57.4	0.9191	49.58
63.3	0.9059	55.47	57.3	0.9193	49.48
63.2	0.9062	55.37	57.2	0.9196	49.38
63.1	0.9064	55.27	57.1	0.9198	49.28
63.0	0.9066	55.17	57.0	0.9200	49.19
62.9	0.9069	55.06	56.9	0.9202	49.09
62.8	0.9071	54.96	56.8	0.9204	48.99
62.7	0.9073	54.86	56.7	0.9206	48.90
62.6	0.9076	54.76	56.6	0.9208	48.80
62.5	0.9078	54.66	56.5	0.9210	48.70
62.4	0.9080	54.56	56.4	0.9213	48.60
62.3	0.9082	54.46	56.3	0.9215	48.51
62.2	0.9085	54.36	56.2	0.9217	48.41
62.1	0.9087	54.25	56.1	0.9219	48.31
62.0	0.9089	54.15	56.0	0.9221	48.21
61.9	0.9092	54.05	55.9	0.9223	48.12
61.8	0.9094	53.95	55.8	0.9225	48.02
61.7	0.9096	53.85	55.7	0.9227	47.92
61.6	0.9098	53.75	55.6	0.9229	47.83
61.5	0.9101	53.65	55.5	0.9231	47.73
61.4	0.9103	53.55	55.4	0.9234	47.63
61.3	0.9105	53.45	55.3	0.9236	47.5
61.2	0.9107	53.35	55.2	0.9238	47.44
61.1	0.9110	53.25	55.1	0.9240	47.34
61.0	0.9112	53.15	55.0	0.9242	47.25
60.9	0.9114	53.05	54.9	0.9244	47.15
60.8	0.9116	52.95	54.8	0.9246	47.05
60.7	0.9119	52.85	54.7	0.9248	46.96
60.6	0.9121	52.75	54.6	0.9250	46.86
60.5	0.9123	52.65	54.5	0.9252	46.76
60.4	0.9125	52.55	54.4	0.9254	46.67
60.3	0.9128	52.45	54.3	0.9256	46.57
60.2	0.9130	52.35	54.2	0.9258	46.47
60.1	0.9132	52.25	54.1	0.9260	46.38
60.0	0.9134	52.15	54.0	0.9263	46.28
59.9	0.9137	52.05	53.9	0.9265	46.19
59.8	0.9139	51.95	53.8	0.9267	46.09
59.7	0.9141	51.85	53.7	0.9269	46.00
59.6	0.9143	51.75	53.6	0.9271	45.90
59.5	0.9145	51.65	53.5	0.9273	45.80
59.4	0.9148	51.55	53.4	0.9275	45.71
59.3	0.9150	51.45	53.3	0.9277	45.61
59.2	0.9152	51.35	53.2	0.9279	45.52
59.1	0.9154	51.25	53.1	0.9281	45.42
59.0	0.9156	51.15	53.0	0.9283	45.33
58.9	0.9159	51.05	52.9	0.9285	45.23
58.8	0.9161	50.96	52.8	0.9287	45.14
58.7	0.9163	50.86	52.7	0.9289	45.04
58.6	0.9165	50.76	52.6	0.9291	44.94
58.5	0.9167	50.66	52.5	0.9293	44.85
58.4	0.9170	50.56	52.4	0.9295	44.75
58.3	0.9172	50.46	52.3	0.9297	44.66
58.2	0.9174	50.36	52.2	0.9299	44.56
58.1	0.9176	50.27	52.1	0.9301	44.47

Sp. Gr. at 60°/60° F. (15.56°/15.56° C.) of Mixtures of Alcohol and Water—  
(Continued)

Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight	Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight
52.0	0.9303	44.37	46.0	0.9417	38.78
51.9	0.9305	44.28	45.9	0.9419	38.69
51.8	0.9307	44.19	45.8	0.9421	38.59
51.7	0.9309	44.09	45.7	0.9423	38.50
51.6	0.9311	44.00	45.6	0.9425	38.41
51.5	0.9313	43.90	45.5	0.9426	38.32
51.4	0.9315	43.81	45.4	0.9428	38.23
51.3	0.9317	43.71	45.3	0.9430	38.14
51.2	0.9319	43.62	45.2	0.9432	38.05
51.1	0.9321	43.52	45.1	0.9433	37.95
51.0	0.9323	43.43	45.0	0.9435	37.86
50.9	0.9325	43.33	44.9	0.9437	37.77
50.8	0.9327	43.24	44.8	0.9439	37.68
50.7	0.9329	43.15	44.7	0.9440	37.59
50.6	0.9331	43.05	44.6	0.9442	37.50
50.5	0.9333	42.96	44.5	0.9444	37.41
50.4	0.9335	42.86	44.4	0.9445	37.32
50.3	0.9337	42.77	44.3	0.9447	37.23
50.2	0.9339	42.68	44.2	0.9449	37.14
50.1	0.9341	42.58	44.1	0.9451	37.05
50.0	0.9343	42.49	44.0	0.9452	36.95
49.9	0.9345	42.39	43.9	0.9454	36.86
49.8	0.9346	42.30	43.8	0.9456	36.77
49.7	0.9348	42.21	43.7	0.9457	36.68
49.6	0.9350	42.11	43.6	0.9459	36.59
49.5	0.9352	42.02	43.5	0.9461	36.50
49.4	0.9354	41.93	43.4	0.9463	36.41
49.3	0.9356	41.83	43.3	0.9464	36.32
49.2	0.9358	41.74	43.2	0.9466	36.23
49.1	0.9360	41.65	43.1	0.9468	36.14
49.0	0.9362	41.55	43.0	0.9469	36.05
48.9	0.9364	41.46	42.9	0.9471	35.96
48.8	0.9366	41.37	42.8	0.9473	35.87
48.7	0.9368	41.27	42.7	0.9474	35.78
48.6	0.9369	41.18	42.6	0.9476	35.69
48.5	0.9371	41.09	42.5	0.9478	35.60
48.4	0.9373	40.99	42.4	0.9479	35.51
48.3	0.9375	40.90	42.3	0.9481	35.42
48.2	0.9377	40.81	42.2	0.9482	35.33
48.1	0.9379	40.71	42.1	0.9484	35.24
48.0	0.9381	40.62	42.0	0.9486	35.15
47.9	0.9383	40.53	41.9	0.9487	35.06
47.8	0.9384	40.44	41.8	0.9489	34.97
47.7	0.9386	40.34	41.7	0.9491	34.88
47.6	0.9388	40.25	41.6	0.9492	34.79
47.5	0.9390	40.16	41.5	0.9494	34.70
47.4	0.9392	40.07	41.4	0.9496	34.61
47.3	0.9394	39.97	41.3	0.9497	34.52
47.2	0.9395	39.88	41.2	0.9499	34.43
47.1	0.9397	39.79	41.1	0.9500	34.34
47.0	0.9399	39.70	41.0	0.9502	34.26
46.9	0.9401	39.61	40.9	0.9504	34.17
46.8	0.9403	39.51	40.8	0.9505	34.08
46.7	0.9405	39.42	40.7	0.9507	33.99
46.6	0.9407	39.33	40.6	0.9508	33.90
46.5	0.9408	39.24	40.5	0.9510	33.81
46.4	0.9410	39.15	40.4	0.9511	33.72
46.3	0.9412	39.05	40.3	0.9513	33.63
46.2	0.9414	38.96	40.2	0.9515	33.54
46.1	0.9416	38.87	40.1	0.9516	33.45

Sp. Gr. at 60°/60° F. (15.56°/15.56° C.) of Mixtures of Alcohol and Water—  
(Continued)

Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight	Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight
40.0	0.9518	33.36	34.0	0.9604	28.10
39.9	0.9519	33.27	33.9	0.9605	28.05
39.8	0.9521	33.19	33.8	0.9607	27.93
39.7	0.9522	33.10	33.7	0.9608	27.84
39.6	0.9524	33.01	33.6	0.9609	27.76
39.5	0.9526	32.92	33.5	0.9611	27.67
39.4	0.9527	32.83	33.4	0.9612	27.59
39.3	0.9529	32.74	33.3	0.9613	27.50
39.2	0.9530	32.65	33.2	0.9614	27.41
39.1	0.9532	32.57	33.1	0.9616	27.33
39.0	0.9533	32.48	33.0	0.9617	27.24
38.9	0.9535	32.39	32.9	0.9618	27.16
38.8	0.9536	32.30	32.8	0.9620	27.07
38.7	0.9538	32.21	32.7	0.9621	26.98
38.6	0.9539	32.12	32.6	0.9622	26.90
38.5	0.9541	32.04	32.5	0.9623	26.81
38.4	0.9542	31.95	32.4	0.9625	26.72
38.3	0.9544	31.86	32.3	0.9626	26.64
38.2	0.9545	31.77	32.2	0.9627	26.55
38.1	0.9547	31.68	32.1	0.9628	26.47
38.0	0.9548	31.60	32.0	0.9630	26.38
37.9	0.9550	31.51	31.9	0.9631	26.30
37.8	0.9551	31.42	31.8	0.9632	26.21
37.7	0.9553	31.33	31.7	0.9633	26.12
37.6	0.9554	31.24	31.6	0.9635	26.04
37.5	0.9556	31.16	31.5	0.9636	25.95
37.4	0.9557	31.07	31.4	0.9637	25.87
37.3	0.9558	30.98	31.3	0.9638	25.78
37.2	0.9560	30.89	31.2	0.9639	25.70
37.1	0.9561	30.80	31.1	0.9641	25.61
37.0	0.9563	30.72	31.0	0.9642	25.52
36.9	0.9564	30.63	30.9	0.9643	25.44
36.8	0.9566	30.54	30.8	0.9644	25.35
36.7	0.9567	30.45	30.7	0.9645	25.27
36.6	0.9568	30.37	30.6	0.9646	25.18
36.5	0.9570	30.28	30.5	0.9648	25.10
36.4	0.9571	30.19	30.4	0.9649	25.01
36.3	0.9573	30.10	30.3	0.9650	24.93
36.2	0.9574	30.02	30.2	0.9651	24.84
36.1	0.9576	29.93	30.1	0.9652	24.75
36.0	0.9577	29.84	30.0	0.9653	24.67
35.9	0.9578	29.76	29.9	0.9655	24.59
35.8	0.9580	29.67	29.8	0.9656	24.50
35.7	0.9581	29.58	29.7	0.9657	24.42
35.6	0.9583	29.49	29.6	0.9658	24.33
35.5	0.9584	29.41	29.5	0.9659	24.25
35.4	0.9585	29.32	29.4	0.9660	24.16
35.3	0.9587	29.23	29.3	0.9661	24.08
35.2	0.9588	29.15	29.2	0.9662	23.99
35.1	0.9589	29.06	29.1	0.9664	23.91
35.0	0.9591	28.97	29.0	0.9665	23.82
34.9	0.9592	28.88	28.9	0.9666	23.74
34.8	0.9593	28.80	28.8	0.9667	23.65
34.7	0.9595	28.71	28.7	0.9668	23.57
34.6	0.9596	28.62	28.6	0.9669	23.48
34.5	0.9597	28.54	28.5	0.9670	23.40
34.4	0.9599	28.45	28.4	0.9671	23.31
34.3	0.9600	28.36	28.3	0.9673	23.23
34.2	0.9601	28.28	28.2	0.9674	23.14
34.1	0.9603	28.19	28.1	0.9675	23.06

Sp. Gr. at 60°/60° F. (15.56°/15.56° C.) of Mixtures of Alcohol and Water—  
(Continued)

Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight	Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight
28.0	0.9676	22.97	22.0	0.9739	17.93
27.9	0.9677	22.89	21.9	0.9740	17.85
27.8	0.9678	22.80	21.8	0.9741	17.77
27.7	0.9679	22.72	21.7	0.9742	17.68
27.6	0.9680	22.63	21.6	0.9744	17.60
27.5	0.9681	22.55	21.5	0.9745	17.52
27.4	0.9683	22.47	21.4	0.9746	17.43
27.3	0.9684	22.38	21.3	0.9747	17.35
27.2	0.9685	22.30	21.2	0.9748	17.27
27.1	0.9686	22.21	21.1	0.9749	17.18
27.0	0.9687	22.13	21.0	0.9750	17.10
26.9	0.9688	22.04	20.9	0.9751	17.02
26.8	0.9689	21.95	20.8	0.9752	16.93
26.7	0.9690	21.87	20.7	0.9753	16.85
26.6	0.9691	21.79	20.6	0.9754	16.77
26.5	0.9692	21.71	20.5	0.9755	16.68
26.4	0.9693	21.62	20.4	0.9756	16.60
26.3	0.9695	21.54	20.3	0.9757	16.52
26.2	0.9696	21.45	20.2	0.9758	16.43
26.1	0.9697	21.37	20.1	0.9759	16.35
26.0	0.9698	21.28	20.0	0.9760	16.27
25.9	0.9699	21.20	19.9	0.97605	16.18
25.8	0.9700	21.12	19.8	0.9761	16.10
25.7	0.9701	21.03	19.7	0.9762	16.02
25.6	0.9702	20.95	19.6	0.9763	15.94
25.5	0.9703	20.86	19.5	0.9764	15.85
25.4	0.9704	20.78	19.4	0.9765	15.77
25.3	0.9705	20.70	19.3	0.9766	15.69
25.2	0.9706	20.61	19.2	0.9767	15.61
25.1	0.9707	20.53	19.1	0.9768	15.52
25.0	0.9708	20.44	19.0	0.9769	15.44
24.9	0.9709	20.36	18.9	0.9770	15.36
24.8	0.9710	20.28	18.8	0.9771	15.27
24.7	0.9712	20.19	18.7	0.9772	15.19
24.6	0.9713	20.11	18.6	0.9773	15.11
24.5	0.9714	20.02	18.5	0.9774	15.03
24.4	0.9715	19.94	18.4	0.9775	14.94
24.3	0.9716	19.86	18.3	0.9776	14.86
24.2	0.9717	19.77	18.2	0.9777	14.78
24.1	0.9718	19.68	18.1	0.9778	14.70
24.0	0.9719	19.60	18.0	0.9779	14.61
23.9	0.9720	19.52	17.9	0.9780	14.53
23.8	0.9721	19.44	17.8	0.9781	14.45
23.7	0.9722	19.35	17.7	0.9782	14.36
23.6	0.9723	19.27	17.6	0.9783	14.26
23.5	0.9724	19.18	17.5	0.9784	14.20
23.4	0.9725	19.10	17.4	0.9785	14.12
23.3	0.9726	19.02	17.3	0.9786	14.03
23.2	0.9727	18.93	17.2	0.9787	13.95
23.1	0.9728	18.85	17.1	0.9788	13.87
23.0	0.9729	18.77	17.0	0.9789	13.79
22.9	0.9730	18.68	16.9	0.9790	13.70
22.8	0.9731	18.60	16.8	0.9791	13.62
22.7	0.9732	18.52	16.7	0.9792	13.54
22.6	0.9733	18.43	16.6	0.9794	13.46
22.5	0.9734	18.35	16.5	0.9795	13.37
22.4	0.9735	18.27	16.4	0.9796	13.29
22.3	0.9736	18.18	16.3	0.9797	13.21
22.2	0.9737	18.10	16.2	0.9798	13.13
22.1	0.9738	18.02	16.1	0.9799	13.04

Sp. Gr. at 60°/60° F. (15.56°/15.56° C.) of Mixtures of Alcohol and Water—  
(Continued)

Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight	Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight
16.0	0.9800	12.96	10.0	0.9866	8.05
15.9	0.9801	12.88	9.9	0.9867	7.97
15.8	0.9802	12.80	9.8	0.9868	7.88
15.7	0.9803	12.71	9.7	0.9870	7.80
15.6	0.9804	12.63	9.6	0.9871	7.72
15.5	0.9805	12.55	9.5	0.9872	7.64
15.4	0.9806	12.47	9.4	0.9873	7.56
15.3	0.9807	12.39	9.3	0.9874	7.48
15.2	0.9808	12.30	9.2	0.9876	7.40
15.1	0.9809	12.22	9.1	0.9877	7.31
15.0	0.9810	12.14	9.0	0.9878	7.23
14.9	0.9811	12.06	8.9	0.9879	7.15
14.8	0.9812	11.97	8.8	0.9880	7.07
14.7	0.9814	11.89	8.7	0.9881	6.99
14.6	0.9815	11.81	8.6	0.9883	6.91
14.5	0.9816	11.73	8.5	0.9884	6.83
14.4	0.9817	11.65	8.4	0.9885	6.75
14.3	0.9818	11.56	8.3	0.9886	6.67
14.2	0.9819	11.48	8.2	0.9887	6.58
14.1	0.9820	11.40	8.1	0.9889	6.50
14.0	0.9821	11.32	8.0	0.9890	6.42
13.9	0.9822	11.23	7.9	0.9891	6.34
13.8	0.9823	11.15	7.8	0.9892	6.26
13.7	0.9824	11.07	7.7	0.9893	6.18
13.6	0.9825	10.99	7.6	0.9895	6.10
13.5	0.9826	10.91	7.5	0.9896	6.02
13.4	0.9827	10.82	7.4	0.9897	5.94
13.3	0.9828	10.74	7.3	0.9898	5.85
13.2	0.9829	10.66	7.2	0.9900	5.77
13.1	0.9831	10.58	7.1	0.9901	5.69
13.0	0.9832	10.50	7.0	0.9902	5.61
12.9	0.9833	10.42	6.9	0.9903	5.53
12.8	0.9834	10.33	6.8	0.9905	5.45
12.7	0.9835	10.25	6.7	0.9906	5.37
12.6	0.9836	10.17	6.6	0.9907	5.29
12.5	0.9837	10.09	6.5	0.9908	5.21
12.4	0.9838	10.01	6.4	0.9910	5.13
12.3	0.9840	9.92	6.3	0.9911	5.05
12.2	0.9841	9.84	6.2	0.9912	4.97
12.1	0.9842	9.76	6.1	0.9914	4.88
12.0	0.9843	9.68	6.0	0.9915	4.80
11.9	0.9844	9.60	5.9	0.9916	4.72
11.8	0.9845	9.52	5.8	0.9918	4.64
11.7	0.9846	9.43	5.7	0.9919	4.56
11.6	0.9847	9.35	5.6	0.9920	4.48
11.5	0.9849	9.27	5.5	0.9921	4.40
11.4	0.9850	9.19	5.4	0.9923	4.32
11.3	0.9851	9.11	5.3	0.9924	4.24
11.2	0.9852	9.03	5.2	0.9925	4.16
11.1	0.9853	8.94	5.1	0.9927	4.08
11.0	0.9854	8.86	5.0	0.9928	4.00
10.9	0.9856	8.78	4.9	0.9930	3.92
10.8	0.9857	8.70	4.8	0.9931	3.84
10.7	0.9858	8.62	4.7	0.9932	3.76
10.6	0.9859	8.54	4.6	0.9934	3.68
10.5	0.9860	8.45	4.5	0.9935	3.60
10.4	0.9861	8.37	4.4	0.9936	3.52
10.3	0.9862	8.29	4.3	0.9938	3.44
10.2	0.9864	8.21	4.2	0.9939	3.35
10.1	0.9865	8.13	4.1	0.9940	3.27

Sp. Gr. at 60°/60° F. (15.56°/15.56° C.) of Mixtures of Alcohol and Water—  
(Continued)

Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight	Percentage of absolute alcohol by volume	Sp. gr. at 60°/60° F.	Percentage of absolute alcohol by weight
4.0	0.9942	3.19	2.0	0.9970	1.59
3.9	0.9943	3.11	1.9	0.9972	1.51
3.8	0.9945	3.03	1.8	0.9973	1.43
3.7	0.9946	2.95	1.7	0.9975	1.35
3.6	0.9947	2.87	1.6	0.9976	1.27
3.5	0.9949	2.79	1.5	0.9978	1.19
3.4	0.9950	2.71	1.4	0.9979	1.11
3.3	0.9952	2.63	1.3	0.9981	1.03
3.2	0.9953	2.54	1.2	0.9982	0.95
3.1	0.9954	2.47	1.1	0.9983	0.87
3.0	0.9956	2.39	1.0	0.9985	0.80
2.9	0.9957	2.31	0.9	0.9986	0.72
2.8	0.9959	2.23	0.8	0.9988	0.64
2.7	0.9960	2.15	0.7	0.9989	0.56
2.6	0.9962	2.07	0.6	0.9991	0.48
2.5	0.9963	1.99	0.5	0.9992	0.40
2.4	0.9964	1.91	0.4	0.9994	0.32
2.3	0.9966	1.83	0.3	0.9995	0.24
2.2	0.9967	1.75	0.2	0.9997	0.16
2.1	0.9969	1.67	0.1	0.9998	0.08
			0.0	1.0000	0.00

does require an accuracy of this order and between 99 and 100% alcohol, the sp. gr. is given to five places. In the same way correction of weights to vacuum is not usually required except with absolute alcohol, where its omission would lead to an apparent alcohol content too high by about 0.05%. Where results of this accuracy are required on alcohols other than "absolute" the Bureau's original tables of five-place sp. grs. should be used. The correction of an apparent weight in air with brass weights to weight in vacuo is made by the following formula:  $WV = WA + (K \times WA/1000)$ , where

$WV$  = weight in vacuum.

$WA$  = apparent weight in air.

$K$  = a number depending on the density of the air, of the weights, and of the body weighed.

Assuming a mean value of 0.0012 for air, and brass weights, 8.4, then  $K$  will have the value +1.36, +1.19 or +1.06, when the density of the body weighed is 0.8, 0.9 or 1.0 respectively (*loc. cit.* B. of S. Circ. No. 19).

Where work of this accuracy is required the equipment must correspond—a water bath with temperature control of the order

indicated above, a tested thermometer reading to  $0.01^{\circ}$  C., a good type of pycnometer, weights carefully tested among themselves, and a balance to suit, being essential.

Some forms of pycnometer can be filled at one temperature and weighted after an interval at another. With such a type one must make very sure of the tightness of the apparatus, and convince himself by actual trial that the evaporation losses are negligible. The ordinary sp. gr. bottle must be weighed immediately it is filled and consequently at the temperature of filling. When as sometimes happens in a chemical laboratory, the dew point is above  $60^{\circ}$  F., the ordinary sp. gr. bottle must be abandoned, or the comparison made at a higher temperature,  $62^{\circ}$  F.,  $64^{\circ}$ , or even higher, and apart from considerations of dew, it is frequently difficult, if not actually impossible to cool liquids to  $60^{\circ}$  F. Consequently the sp. gr. may be determined at a temperature higher than  $60^{\circ}$  F. Since the coefficient of expansion of alcohol is much greater than that of water, the sp. gr. at  $62^{\circ}/62^{\circ}$  is widely different from the sp. gr. at  $60^{\circ}/60^{\circ}$  and a correction must be made. If the Bureau of Standards publications are at hand one has sufficient information for an exact conversion to  $60^{\circ}/60^{\circ}$  basis of a sp. gr. determined at any other likely temperature. Where these are not available the following table of approximate corrections given in the IV edition, will prove useful where water and alcohol have both been weighed at the same temperature  $T$ , above  $60^{\circ}$  F. It shows the amount to be added to the observed sp. gr. at  $T/T$ , for each degree Fahrenheit by which  $T$  exceeds  $60^{\circ}$ . Since alcohol expands unequally for equal increments of temperature, the correction is less accurate the farther removed  $T$  is from  $60^{\circ}$ , hence,  $T$  should be kept as near to  $60^{\circ}$  as possible.

ADDITIONS TO BE MADE TO SP. GR. OF  
AQUEOUS ALCOHOL OBSERVED AT 61°/61°F.

In order to convert them into sp. gr. at 60°/60° F.

Sp. gr. at 61°/61° F.	Addition	Sp. gr. at 61°/61° F.	Addition
0.794 to 0.860	0.00039	0.963 to 0.964	0.00019
0.860 to 0.890	0.00038	0.964 to 0.966	0.00018
0.890 to 0.905	0.00037	0.966 to 0.967	0.00017
0.905 to 0.916	0.00036	0.967 to 0.968	0.00016
0.916 to 0.925	0.00035	0.968 to 0.969	0.00015
0.925 to 0.932	0.00034	0.969 to 0.970	0.00014
0.932 to 0.937	0.00033	0.970 to 0.971	0.00013
0.937 to 0.941	0.00032	0.971 to 0.972	0.00012
0.941 to 0.944	0.00031	0.972 to 0.973	0.00011
0.944 to 0.947	0.00030	0.973 to 0.974	0.00010
0.947 to 0.949	0.00029	0.974 to 0.975	0.00009
0.949 to 0.951	0.00028	0.975 to 0.976	0.00008
0.951 to 0.953	0.00027	0.976 to 0.977	0.00007
0.953 to 0.954	0.00026	0.977 to 0.979	0.00006
0.954 to 0.956	0.00025	0.979 to 0.981	0.00005
0.956 to 0.958	0.00024	0.981 to 0.982	0.00004
0.958 to 0.959	0.00023	0.982 to 0.984	0.00003
0.959 to 0.961	0.00022	0.984 to 0.986	0.00002
0.961 to 0.962	0.00021	0.986 to 0.994	0.00001
0.962 to 0.963	0.00020		

The following formulas may be used for converting percentage of alcohol by weight or volume to per cent. of English proof spirit (defined in an earlier paragraph) and *vice versa*. The per cent. of American proof spirit is equal to twice the per cent. of alcohol by volume.

If  $W$  is per cent. by weight,  $V$ , per cent. by volume,  $P$ , the percentage of proof spirit, and  $D$  the sp. gr. at 60°/60° F., then the following equations hold:

$$V = P \times 0.5706, \text{ or } \frac{WD}{0.7938} \text{ or } WD \times 1.26$$

$$P = \frac{V}{0.5706} \text{ or } V \times 1.7525, \text{ or } P = WD \times 2.208$$

$$W = \frac{P}{D \times 2.208}, \text{ or } W = V \times \frac{0.7938}{D}$$

The following are examples of calculations, such as have frequently to be made in British practice:

If it be required to know what percentage of gin at 20° U.P. is contained in a watered sample of 44° U.P., the following calculation will suffice.

$$\frac{(100 - 44) 100}{100 - 20} = \frac{56 \times 100}{100} = 70\% \text{ by volume.}$$

Hence the sample is of a strength corresponding to the dilution of 7 gallons of gin at 20° U.P. to 10 gallons by the addition of water.

Again, to ascertain the proportion of water which must be added to spirit at 35° O.P. to reduce the strength to 10° U.P.:

$$\frac{(100 - 10) 100}{100 \div 35} = \frac{90 \times 100}{135} = 66.7$$

That is, to obtain spirit of 10° U.P., 66.7 volumes of spirit at 35° O.P. must be diluted to 100, or every 2 gallons must be made up to 3 by addition of water.

*The estimation of alcohol in the presence of fixed matters* is usually effected by distillation of the sample and ascertaining the sp. gr. of the distillate. It is sometimes necessary, and generally advisable, to neutralize the liquid before distillation, but this must not be done when ascertaining the original gravity of beers. With carbonated liquids, the free carbon dioxide is first expelled by pouring back and forth from one beaker to another, removing the froth from time to time, the operation being repeated until the liquid is quiet and shows little tendency to foam.

The quantity to be taken will depend on the alcoholic strength of the sample, and is sometimes conditioned by the small quantity supplied to the analyst. 100 c.c. (or 100 grm., if weight per cent. is required) is a convenient quantity of beer, wines, or cider. The beer should be distilled till about 80 c.c. has come over, and the distillate should be made up with distilled water to 100 c.c. In the case of wines it is better to add 50 c.c. of water and a little tannin to 100 c.c. of the wine, and to distil until nearly 100 c.c. have been collected. Of potable spirit which contains about 50% of alcohol, it is convenient to mix 50 c.c. with 100 c.c. of water and to distil over about 100 c.c. Stronger spirit should be still further diluted, 25 c.c. being diluted to 150 c.c. with water and 100 c.c. distilled. The distillate in any case is made up to 100 c.c. at 60° and its sp. gr. determined at 60°/60° F. Reference to the tables will give at once either per cent. alcohol by volume or weight per cent. in the distillate. Then:

$$\frac{\text{Sp. gr. of distillate} \times \text{c.c. distillate} \times \text{wt. per cent. in distillate by table}}{\text{Sp. gr. of sample} \times \text{c.c. of sample taken}}$$

gives the percentage of absolute alcohol by weight contained in the sample.

This calculation involves the necessity of knowing the sp. gr. of the *original sample*. If unknown, the 50 c.c. or 100 c.c. taken for the experiment may be accurately weighed, and this weight in grams substituted for the denominator of the above fraction.

The calculation can be wholly avoided and a more satisfactory result obtained by *weighing* the original sample instead of measuring it, and also weighing the distillate. Then:

$$\frac{\text{Weight of distillate} \times \text{wt. per cent. in distillate by table}}{\text{Weight of sample taken}} \text{ gives the}$$

percentage of absolute alcohol by weight contained in the sample.

The percentage by volume in the original is obtained by multiplying the per cent. by volume in the distillate by 100 and dividing the result by the volume of the original taken for distillation.

The following indirect method is less accurate than the distillation method but is sometimes useful. The sp. gr. of the original liquid is first taken, and then 100 c.c. are evaporated to expel alcohol and subsequently made up again with distilled water to 100 c.c. at 60° F. and the sp. gr. of the alcohol-free liquid determined. Let this be  $S_2$  and the sp. gr. of the original liquid  $S_1$ . From these numbers it is possible to calculate with fair accuracy what would have been the sp. gr.,  $s$ , of the distillate supposing the liquid had been distilled and the distillate made up to 100 c.c., as follows:

$$S = 1 + S_1 - S_2$$

From the value of  $S$  thus found, the percentage of alcohol may be obtained by reference to the tables already given.

The alcoholic strength of *potable spirits*, which, with the exception of gin, rarely contain more than 0.5% of solid matter, may be approximately ascertained from the sp. gr. of the original spirit and the proportion of the solid matter. If the spirit has a sp. gr.  $S$ , and contains  $W$  grm. of solid matter per 100 c.c., then:

$S = S_1 - 0.0055 W$ , where  $S$  has the same significance as in the last paragraph.

The *refractometer* may be applied to the estimation of alcohol in liquids consisting solely of alcohol and water. In the presence of

fixed matters, resort must be had to distillation, or to an indirect method exactly analogous to the indirect sp. gr. method. A table for use with the Zeiss immersion refractometer has been already given in the section on Methyl Alcohol. A more extended one is that of Wagner and Schultze (*Zeit. anal. Chem.*, 1907, **46**, 508) and Wagner, who from time to time, in the *Chemiker Zeitung* and elsewhere, published sections of the table in greatly extended form, with convenient temperature corrections for special purposes, brought out a book *Tabellen zum Eintauchre-fraktometer*, Carl Zeiss, Jena, 1903) to which readers are referred for further information. More recent tables are those calculated and rearranged by B. H. St. John from the data of Doroshevskii and Dvorzhanchik (*J. Russ. Phys. Chem. Soc.*, 1908, **40**, 107) and published in the *J. of the Assoc. of Off. Agric. Chem.*, 1916, **2**, 208 and reprinted in its *Official and Tentative Methods of Analysis*, revised to 1919, published at Washington in 1920. This 27-page table gives weight per cent. and volume per cent. alcohol corresponding to every  $\frac{1}{10}$  division Zeiss scale reading between 15 and 80 at 17.5°, 18°, 19°, 20°, 21°, 22°, 23°, 24° and 25°.

The refractometer is particularly useful in estimating methyl and ethyl alcohols in mixtures, but for the estimation of ethyl alcohol in aqueous solution it is a less accurate instrument than the pycnometer, and if it is desired to obtain results of any value by its means, so much attention must be paid ordinarily to the temperature that its use can hardly be recommended on the score of speed. However given a laboratory properly equipped for careful temperature control and for handling many analyses at a time the method requires decidedly less time and manipulation than the sp. gr. method. A discussion of the instrument and its manipulation is given on pages 23 to 33 of the Introduction.

**Boiling Point Method.**—This method has been developed considerably of late, especially in France, where it is used for official purposes. A late form of the apparatus, a proprietary design described by Cain (*Chem. News*, 1914, **109**, 37) is costly, but it is said to give results of great exactness, and is operated simply, consequently it may be expected to find increasing use in laboratories where large numbers of alcohol estimations must be made. One of the simplest and most convenient types is the Salleron ebulliometer which is used occasionally in distilleries in the United States, and which consists of

a jacketed container made of metal, heated from underneath by a lamp, fitted with a reflux condenser and provided with a delicate thermometer reading to  $\frac{1}{10}^{\circ}$ . The instrument is fitted with an adjustable three-scale attachment, the middle scale of which, graduated

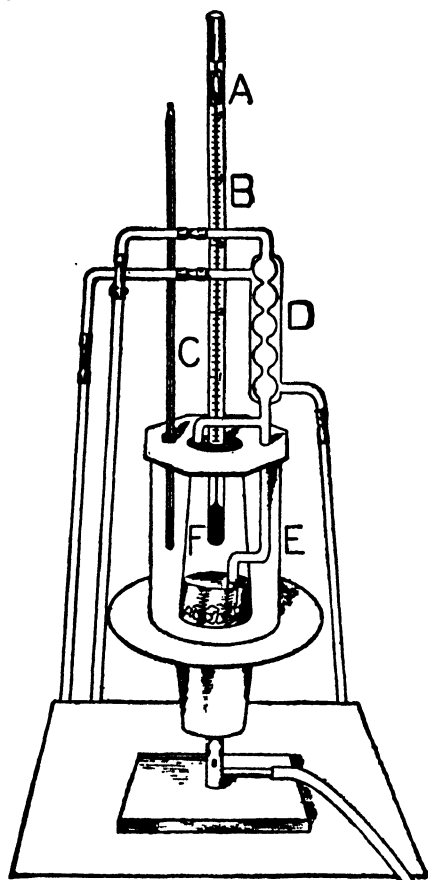


FIG. 54.

in  $\frac{1}{10}^{\circ}$  C. slides up and down between two fixed scales, one graduated to show per cent. of alcohol corresponding to b. p. in case of mixtures of alcohol and water, the other graduated to show in the same way the per cent. alcohol corresponding to temperature for mixtures of a corresponding range of alcohol per cent. but with considerable residue present (amounts normal to the product being examined, cider, beer, wine, etc). If the instrument is kept adjusted to b. p. changes resulting from changes in barometric pressure, and if the third scale of the instrument is carefully calibrated by known alcohol-water solutions of the solids characteristic of the fermented mash on which it is to be used, it is said to give in distillery control work results equal to those by sp. gr. and in shorter time.

Wiley has described a form of apparatus which can be assembled by the chemist himself and which is claimed to be quite accurate. It consists of the flask *F*, closed by a rubber stopper, carrying a differential thermometer *B* (grad. in  $0.01^{\circ}$  C.) and a vapour tube leading to condenser *D*. The condensed vapours are returned outside the flask, as indicated in the figure, and enter below the liquid level.

The flask is heated by a gas lamp and is placed upon an asbestos disc in such a way as to cover entirely the hole in the centre of the disc, which is a little smaller than the bottom of the flask. The whole apparatus is protected from external influences of temperature by the glass cylinder, *E*, which rests upon the asbestos disc below and is covered with a detachable stiff rubber cloth disc above. The thermometer, *C*, indicates the temperature of the air between *F* and *E*. *B* should always be read with *C* indicating the same air temperature. The tube leading from the condenser tube, *D*, to the left is made long and is left open at its lower extremity in order to maintain atmospheric pressure in *F* and at the same time reduce diffusion of alcoholic vapours through *D*.

The heat is so regulated that *C* reads about  $90^{\circ}$  in 10 minutes, with solutions 5% or less. When *B* becomes stationary (a few minutes of boiling) readings are taken (to  $0.005^{\circ}$  by means of a cathetometer) at 30 second intervals for 10 minutes. Pieces of scrap platinum are placed in the flask to prevent bumping and secure a more uniform vapour temperature. Surrounding the bulb of the large thermometer with a test-tube containing water, protects *B* against liquor spray and equalizes the temperature so that oscillations of the mercury column are minimized.

The thermometer *B* is of the usual Beckmann type used for molecular weight determinations by the b. p. method. The thermometer is set in the first place by putting the bulb in water containing 16 grm. of common salt per 100 c.c. and heating. When the water is fully boiling, the excess of mercury is removed from the column in the receptacle, at the top. Then, on placing in boiling water, the column of mercury will be found a little above the  $5^{\circ}$  mark (thermometer with  $5^{\circ}$  range used). This setting will allow a variation in all of  $5^{\circ}$  in the temperature and is suitable for the estimation of percentages of alcohol from 1 to 5.5 by volume. Stronger solutions are diluted to these limits.

In order to avoid frequent checking of the thermometer, rendered necessary by changes in barometric pressure, a second apparatus, made exactly like the one described, is used, in which water is kept constantly boiling. It is necessary in this case only to read the two thermometers at the same instant in order to obtain the reading corrected for changes in the barometric pressure.

Wiley gives no table relating per cent. alcohol to depression in vapour temperature but states that from 0 to 5% by volume the curve showing the variation in depression is practically a straight line, each  $0.8^{\circ}$  C. change in temperature corresponding to about 1% alcohol by volume. For example, if, in a given case, the temperature of the vapour of boiling water, as marked by the thermometer, is  $5.155$  and that from a sample of beer is  $2.345^{\circ}$ , the depression is equivalent to  $2.810$ , and the percentage of alcohol by volume is, therefore,  $2.81$  divided by  $0.80 = 3.51$ .

*Freezing-point Method.*—Alcohol in aqueous solution can be estimated with a fair degree of accuracy by observing the depression of the freezing point in Beckmann's apparatus, provided the alcohol does not exceed 7%. Below that strength, the depression of the freezing point is approximately proportional to the percentage of alcohol, being, according to Gaunt (*Zeit. anal. Chem.*, 1905, **44**, 106) 0.425 for each 1%. The method is said by Gaunt to be quicker than the sp. gr. method.

*Oxidation Methods.*—The use of chromic acid or permanganate oxidation to determine alcohols has been proposed in a great variety of ways, some of which may be extremely useful, as for example, Hoepner's method for acetone, aldehyde and alcohol, referred to under Denatured Alcohol. Szeberenyi's application (*Zeit. anal. Chem.*, 1915, **54**, 409) to the determination of alcohol in ether, Hetper's method (*Zeit. Nahr. Genussm.*, 1913, **26**, 342) for determining small amounts of methyl and ethyl alcohols, and Budde's method (*Apoth. Ztg.*, 1913, **28**, 580) for alcohol in chloroform, are examples.

*Other Methods.*—The method using the critical temperature of solution with kerosene was mentioned under Absolute Alcohol. Numerous other procedures of the same type have been proposed (*Cf. Lévêque, Bull. soc. pharmacol.*, 1922, **28**, 549, 554) for the examination not only of absolute but of 95% and lower strengths also, but all seem to have found limited or little use. Other alcohol methods have been based on the rate of dilatation by heat, on the surface tension of the liquid, and on the tension of its vapour. These methods are capable of being used with advantage under special circumstances, but they require special apparatus and are generally less accurate and convenient than those already given.

*The estimation of alcohol in essences, tinctures* and other preparations containing substances volatile with alcoholic steam presents

difficulties which may in most cases be surmounted by having recourse to the following method, due to Thorpe and Holmes (*Trans. Chem. Soc.*, 1903, 83, 314):

"25 of the sample, measured at 60° F. are mixed with water in a separator to a volume of from 100 to 150 c.c. and sodium chloride is added in quantity sufficient to saturate the liquid. The mixture is now shaken vigorously for 5 minutes with from 50 to 80 c.c. of petroleum spirit, boiling below 80° and after standing for about half an hour, the lower layer is drawn off into another separator, extracted, if necessary, a second time with petroleum spirit, and then drawn off into a distillation flask. Meanwhile the layers of petroleum spirit are washed successively with 25 c.c. of saturated sodium chloride solution, and the washings added to the main volume, which is neutralized if necessary, and then distilled and the distillate made up to 100 c.c.

"The method, as described, is applicable to preparations containing ether, chloroform, benzaldehyde, and esters. In the greater number of cases, for example, essences of lemon, juniper, peppermint and santal oil preparations, a single extraction is sufficient.

"In the case of all preparations containing camphor, 25 c.c. of normal sulphuric acid are used instead of sodium chloride and a single extraction with petroleum is made. Before distilling it is desirable to neutralize with sodium hydroxide, and if the volume of the liquid becomes inconveniently large some sodium chloride is also added. In preparations containing ammonia this is inadmissible, and the liquid to be distilled must be slightly acid."<sup>1</sup>

<sup>1</sup> NOTE.—The requirement of the U. S. (Federal) food-law and of some state food-laws, that medicinal preparations must bear on the label a statement of the percentage of alcohol present renders it necessary for manufacturers to verify the amount in preparations when finally prepared for sale. C. E. Vanderkleed (*Amer. Jour. Pharm.*, 1909, 89, 129) has made a special study of this phase of the problem and recommends the following method:

50 c.c. of the preparation, measured at a known temperature, are transferred (in portions, if necessary) to a test-tube having an inside diameter of 22 mm. and a height of 200 mm. marked at 50 c.c. The tube is heated in the water bath until all alcohol is driven off. The liquid is cooled to the original temperature, and U.S.P. alcohol (see page 131) at the same temperature is run in from a burette until an amount has been added which when diluted with water to exactly 50 c.c. would give the same alcoholic strength as the menstruum that was used in manufacture of the preparation being assayed. The tube is stoppered, the contents mixed and the sp. gr. ascertained (Vanderkleed uses a Westphal balance). Subtract algebraically the original sp. gr. from that of the solution obtained in the process, and subtract this remainder from the theoretical dilution above noted, and ascertain from the standard tables the percentage of alcohol.

Vanderkleed assayed fluid extracts of Buchu, Cubeb and Santal of known composition and found that the simple distillation method and the method of Thorpe and Holmes gave lower results than the above method. Inasmuch as these complex medicinal preparations are liable to furnish a distillate that contains other substances than alcohol, Vanderkleed does not regard the refraction method as offering any advantage in the solution of this special problem.—H. L.

When applied to the determination of alcohol in preparations containing acetone (as in U.S.P. IX) the extraction procedure above tends to leave considerable and variable amounts of acetone in the water layer. The error seems to be dependent somewhat on the grade of petroleum spirit used.

*The estimation of ethyl alcohol in fusel oil* is sometimes required, since fusel oil containing less than 15% of proof spirit is admitted duty free into the United Kingdom. Thorpe and Holmes have shown that the method, above described, enables them to estimate the ethyl alcohol accurately in a mixture of 73 alcohol, 20 fusel oil and 7 water. If the proportions are 8 alcohol, 90 fusel oil and 2 water, it may be necessary to use rather more petroleum spirit and less sodium chloride solution, but otherwise the method is presumably applicable. Peters (*Pharm. Centralh.*, 1905, **46**, 563) has described a method, similar to that of Thorpe and Holmes, but more complicated. The fusel oil is shaken with water and petroleum spirit and the aqueous layer distilled. The distillate is then shaken with light-petroleum spirit and calcium chloride solution and the aqueous layer again distilled.

Formerly fusel oil was tested by the Excise by shaking it with an equal volume of water to remove the spirit, and then ascertaining the amount of alcohol contained in the aqueous liquid by taking its sp. gr. and noting its volume. The test gives erroneous results, as fusel oil is a mixture of alcohols, of which only amyl alcohol is approximately insoluble in water. As an improvement on this test, G. L. Ulex (*Neues Jahrb. der Pharm.*, **39**, 333) recommended the following, based on the low temperature at which ethyl alcohol distills: 100 c.c. of the sample are heated in a retort until 5 c.c. have passed over, the distillate is shaken with an equal volume of a saturated solution of sodium chloride, and the mixture allowed to stand. If the fusel oil which separates amounts to one-half of the distillate or more, the sample is sure to contain less than 15% of spirit, and is free from any fraudulent admixture with the same. If less fusel oil, or none at all, separates, the presence of 15% of the spirit may be safely assumed. In the latter case, the quantity of the adulterant may be ascertained by shaking a known measure of the sample with an equal volume of a saturated solution of sodium chloride (in which propyl and butyl alcohols are much less soluble than in water), allowing the aqueous

liquid to settle out, distilling it, and estimating the contained alcohol by noting the volume and sp. gr. of the distillate.

Allen showed the accuracy of another method of approximately separating amyl alcohol from ethyl alcohol, which is to agitate the sample in a graduated tube with an equal volume of benzene or petroleum spirit, subsequently adding sufficient water to cause the benzene to separate. The increase in the volume of the benzene indicates with approximate accuracy the amount of amyl alcohol in the sample under examination.

The United States Bureau of Internal Revenue (Regulations No. 61, p. 30, Industrial and Denatured Alcohol, under Title III of the National Prohibition Act of Oct. 28, 1919) before passing a fusel oil as merchantable, *i. e.*, containing so little alcohol as to remove all practical possibility of recovering it—requires that not over 10% shall be soluble in saturated sodium chloride solution. The official procedure for testing is as follows: “the oil must first be thoroughly washed and purified and before being removed from the storage tank must be well mixed and a sample drawn from such tank into a test-tube . . . of glass, bulb-shaped, and closed at one end, having a graduated scale marked upon the glass in degrees from 0 near the top to 100 near the swell of the bulb. The bulb will contain three times as much liquid as that portion of the tube which is graduated from 0 to 100.

“The tube having been filled with saturated salt solution up to the mark 100, oil shall be added until the tube is filled to the mark 0. The oil and saturated salt solution shall then be thoroughly mingled by violently agitating the contents of the tube. If, after sufficient time has been allowed for the oil to fully separate from the salt solution and resume its position at the top of the tube, the scale shall show that not more than 10° or 10% of the oil has disappeared or been dissolved in the salt solution, the oil shall be passed as merchantable . . . but if over 10° of oil disappears the oil will not be considered as sufficiently purified, and cannot be removed in that condition.”

## HIGHER ALIPHATIC ALCOHOLS

The products of commercial importance under this heading are crude and refined fusel oil, normal and isopropyl, normal and iso-

butyl and amyl alcohols. Except for crude and refined fusel oil, the production of these is a recent development in the United States.

**Fusel Oil and the Alcohols from It.**—Crude fusel oil obtained as a distillery by-product varies greatly according to the raw material used for alcohol production, to fermentation conditions, and to the manner of its separation. The proportion of ethyl alcohol plus water is, for average crudes, practically fixed by the Government regulations in force as to the passing of it with respect to ethyl alcohol content (see above). The yields of the various alcohols according to present refining practice may be fairly represented by the following approximate recoveries from four months refining of mixed crudes, both import and domestic, but mainly from Cuban and Porto Rican “black strap” molasses:

Higher alcohols than amyl.....	about	1%
Amyl alcohol.....	about	70%
Isobutyl.....	about	6%
N-propyl.....	about	5%
Balance—ethyl alcohol and water		

By more thorough fractionation the percentage of isobutyl probably could have been slightly increased at the expense of the amyl alcohol fraction. As to the nature of the “higher alcohols” fraction, little is known except that a very considerable part consisted of a mixture of very high boiling alcohols, accumulating especially in the neighborhood of 230°. Occasionally, particularly with imported oils, one finds much larger percentages of the “higher alcohols” fraction than the 1% given above. This is especially true of the crudes obtained from the fermentation of Hawaiian molasses on the West Coast of the United States. It is stated that the fusel oil from this source contains 20 to 30% of products boiling above amyl and in the neighborhood of 270°. The high-boiling constituents here, as above, have not been identified, but they may consist in part at least of a high-boiling fatty acid ester rather than of a high-boiling alcohol and may contain high-boiling hydrocarbons.

Aside from the government alcohol test referred to above, the examination of a crude fusel oil usually includes the following:

(a) *Colour*.—Foreign crudes are apt to be quite coloured, partly due to aging of impurities, partly due to containers.

(b) *Odour*.—This varies more or less according to the source, whether from molasses, grain, potatoes, etc. The fusel oil from the fermentation of Cuban molasses usually has a less disagreeable odour than that from Hawaiian.

(c) *Sp. Gr. at 60°/60° F.*—The range is from 0.830 to 0.850.

(d) *Water content*, as observed from the decrease in volume on saturating with dry potassium carbonate. This gave an average of about 7.5% for the group referred to below.

(e) *Benzine, Kerosene, or Other Non-alcoholic Oils*.—1 vol. of the fusel oil is cautiously mixed with 2 vols. concentrated sulphuric acid in a graduated tube and the undissolved hydrocarbon measured after standing for 1 hour. Fusel oil consisting only of amyl and the lower alcohols separates no clear layer within this time.

(f) *Boiling Range*.—This is made by the direct distillation of 100 c.c. from an Engler flask according to the Bureau of Mines distillation method for gasoline (*Bureau of Mines, Report of Comm. on Stand. of Petroleum Specif., Bull. No. 5. 1921, p. 6*).

Examined according to (f) 25 miscellaneous crudes (half of them imported) gave an average of 32% below 110° and 95% under 135°. The average sp. gr. was 0.839 at 60°/60°; the government ethyl alcohol test 10%, and water by potassium carbonate, 7.5%. One showed 20% hydrocarbons, another 1.3% and the others, none. Of higher boiling material than amyl alcohol, only one (that showing 20% in the sulphuric acid test) had a boiling range like that of Hawaiian crude. The insertion in purchase specifications of an upper b. p. limit, such as 95% recoverable at 140°, would provide ample protection against an unreasonably high percentage of these high-boiling portions which at present are much less valuable than the amyl fraction.

For exact information as to the percentages of the different alcohols present in a given crude, the only reliable procedure seems to be very thorough laboratory fractionation of a large quantity (several litres) of oil, which has been entirely freed from water.

Tests so tedious are seldom warranted however and it is usually sufficient to make a laboratory distillation with 1 to 5 litres, in which the dehydration, distillation, etc., is made to follow as closely as possible the steps of the refinery operations.

For judging the degree to which the refined alcohols have been freed from their homologues, the sharpness of the b. p. gives the simplest indication. In a case of sufficient importance to justify the work involved, much information could be gained without doubt by applying the Duclaux distillation to the volatile acids produced on oxidizing the alcohols by means of chromic acid. (Cf. Katz, *Journ. Assoc. Off. Agric. Chem.*, 1917, 2, 188.)

The crudes are worked up chiefly into N-propyl and isobutyl alcohols and refined fusel oil; amyl alcohol, if desired, is obtained by redistillation of refined fusel oil. When sold for solvent purposes it is generally required of all these products that they be: (a) water-white, (b) water-free, to the extent of remaining clear with at least 5 vols. of gasoline, (c) neutral to moistened red and blue litmus paper (or a titration method, with phenolphthalein indicator, may be used after diluting with water or dilute alcohol), (d) free from aldehydes, as shown by the development of only negligible discolouration when 10 c.c. are shaken with 5 c.c. of ammoniacal 10% silver nitrate solution and allowed to stand in the dark for 30 minutes, and (e) free (in case of refined fusel oil and amyl alcohol) from such nitrogen compounds as develop yellow colour when 15 c.c. of the oil are shaken with 2 c.c. of concentrated nitric acid and observed after 5 and after 30 minutes. There should be no appreciable increase of yellow colour in this interval. Both (d) and (e) are intended to protect against discolouration in the making of films.

Traces of allyl alcohol if produced in the fermentation tend to be eliminated along with the fusel oil in alcohol distillation and when the fusel oil is refined, accumulate in the normal propyl fraction, where the amount may reach 3% or more. The pungent odour and marked physiological action of some of the fusel oil products are probably due almost entirely to their allyl content. This impurity is usually not removed from the commercial grades, but the refined alcohols should be practically free from it. A well-refined N-propyl alcohol for instance should not show more than 0.05% allyl alcohol by the bromine test applied as directed under estimation of wood naphtha, p. 111.

Refined fusel oil, sp. gr. 0.810 to 0.815 at 60°/60° F., is produced, as the trade demands, in different grades depending on the degree to which alcohols lower than amyl are removed; e. g., "60% above 115," "70% above 120°" are typical.

The sp. gr. and boiling range of the alcohols marketed is shown by the following, the sp. gr. of the commercial product being practically the same as that of the refined.

	Sp. gr. 60°/60° F.	Boiling range degrees
Amyl alcohol, refined.....	0.813 to 0.815	128 to 132
Amyl alcohol, commercial.....	.....	120 to 132
Isobutyl alcohol, refined.....	0.807	106 to 108
Isobutyl alcohol, commercial.....	.....	101 to 109
N-propyl alcohol, refined.....	0.809	96 to 98
N-propyl alcohol, commercial.....	.....	90 to 101

By the term refined amyl alcohol is meant the naturally occurring mixture of the isoamyl and active amyl isomers, there being no reason for attempting the separation of the two commercially.

**Isopropyl alcohol** is obtained commercially from the propylene formed as a by-product in the production of "cracked" gasoline, the propylene being absorbed in sulphuric acid and recovered in the form of isopropyl alcohol on diluting and distilling. The process may be applied in the same way to the recovery of the propylene content of natural gas. The crude isopropyl alcohol produced has a very disagreeable, hydrocarbon-like odour, difficult of removal. Produced cheaply enough this crude alcohol may be used in much the same way, as solvent, etc., as denatured alcohol is. Refined to remove the objectionable odours, it is being used in considerable quantity as an alternative for acetone in modified specially denatured alcohols Nos. 39, 39a and 40 (*Treas. Dept. Pro. Mim.* 2800; *Pro.* No. 187, 6/10/21) for use in perfumes, barbers' supply preparations, and toilet preparations.

Isopropyl alcohol for the latter purpose must have a sp. gr. of not more than 0.82130 at 60°/60° F. This is equal to approximately 0.8170 at 20°/4°, which by Lebo's isopropyl alcohol table (*J. Am. Chem. Soc.*, 1921, 43, 1005) corresponds to 90.3% by volume, and 87% isopropyl by weight. The pure alcohol boils at 82.4° at 760 mm. and forms an azeotropic mixture with water, distilling at 80.40° C., containing 87.7% alcohol by weight (91.09% by volume) and having a sp. gr. 0.8158 at 20°/4°. The usual commercial product is a wet alcohol boiling at 80.4 to 81.2°, having a sp. gr. lower than 0.8213 at 60°/60° F.

The refined, dehydrated grade has sp. gr. not over 0.7930 at 60°/60°, corresponding to 98.5% by weight or 98.7% by volume. The permanganate time test as applied to ethyl alcohol may be used equally well in judging refined isopropyl alcohol, thoroughly purified isopropyl showing nearly as high a permanganate time as the best grades of ethyl. The refined grade in particular should be well freed of odourous impurities, as shown by making a 1% solution in the best available cologne spirits and examining odours of the solution and the original cologne spirits side by side according to the odour tests described under the examination of commercial alcohols.

The test prescribed for the identification of isopropyl alcohol is that of Mulliken (*Ident. of Org. Compounds*, I, p. 170, Tests 818) in which the isopropyl alcohol is oxidized to acetone by means of chromic-sulphuric acid mixture. The acetone is distilled out and condensed with benzaldehyde, whereby di-benzylidene-acetone, pale yellow, lustrous plates that melt at 111° to 112° (uncor.), is obtained. Reid (*J. Am. Chem. Soc.*, 1917, **39**, 1255) identifies by converting to isopropyl p-nitro-benzyl-phthalate melting at 74.0°.

**Normal Butyl Alcohol.**—The fermentation process for the production of acetone from starch, developed during the war, yields, in addition to the acetone, about twice its volume of normal butyl alcohol together with a small proportion of ethyl alcohol. The butyl alcohol from this source is obtained in practically pure condition without difficulty, since, unlike the case of isobutyl, there seems to be little or none of the adjacent homologues to complicate the purification.

Commercially, the alcohol is being used as a solvent, as such and in the form of esters, in "dopes" and lacquers in the same way as refined fusel oil is used and from which it differs chiefly in having a considerably lower b. p. The use of it has recently been authorized in the U. S. (*Treas. Dept. Pro. Min.* 2872, 12/5/21) as a special denaturant (formula No. 44) for alcohol to be used in spirit varnishes, varnish removers, and similar preparations, for which purpose it must meet the following specifications: (a) colourless; (b) acidity, less than 0.03% calculated as acetic acid; (c) must give no turbidity when mixed with 19 vols. of pure coal tar benzene; (d) the sp. gr. must be 0.810 to 0.815 at 20°/20°.

The alcohol may be identified by converting into N-butyl-3, 5-dinitro-benzoate, m. p. 64° (uncorr.) (Mulliken I, 163) or to N-

butyl p-nitrobenzyl-phthalate, m. p.  $62.0^{\circ}$ , as recommended by Reid. (*J. Am. Chem. Soc.*, 1917, **39**, 1251).

Young in his recent book on *Distillation Principles and Processes* (1922), Chap. XXV, gives valuable information as to the properties of the pure N-butyl alcohol and its mixtures with acetone and water, and methods of analysis suitable for examination of these mixtures. The following information is chiefly from this source.

Pure N-butyl alcohol boils at  $117.6^{\circ}$  at 763 mm. and has sp. gr. 0.80974 at  $20^{\circ}/4^{\circ}$ . With water it forms an azeotropic mixture, boiling at  $92.25^{\circ}$  containing approximately 37% water by weight, which mixture on cooling separates, leaving an alcohol layer with 80% of alcohol by weight at  $20^{\circ}$ . In practice this 80% alcohol is further dehydrated by saturation with sodium chloride, the alcohol drawn off containing not over 10% of water and being sold as 90% butyl alcohol by weight.

Young gives a table of sp. gr. at  $20^{\circ}/4^{\circ}$  for mixtures of the alcohol with water alone, and also tables and diagrams from which it is possible with any mixture of N-butyl alcohol, acetone and water to estimate all three from a determination of the sp. gr. and of either the acetone or the alcohol.

The critical temperature of solution of the ternary mixture of the alcohol with hydrochloric acid is, according to Orton and Jones, very sensitive to impurities and suitable for the determination of its purity (*Trans. Chem. Soc.*, 1919, **116**, 1194).

For actual estimation of the alcohol Young recommends the acetylation method of Verley and Bolsing (*Ber.*, 1901, **34**, 3354). A known weight of the mixture is treated with anhydrous sodium sulphate in proportion to the water present (approx. estimated from density) and the mixture extracted several times with xylene. The hydrocarbon extract is made up to known volume and a measured amount heated gently on a sand bath, with an excess of a pyridine solution of acetic anhydride, contained in a large flask fitted with a reflux condenser. 2 hours' heating is usually sufficient to complete the esterification. The excess of anhydride is then estimated in the usual way and the butyl alcohol content determined from the amount used.



# MALT AND MALT LIQUORS

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By JULIAN L. BAKER, F. I. C.

Malt is prepared by steeping barley or other grain in water, and allowing it to germinate, the sprouted grain being subsequently dried and cured in a kiln. During these operations the composition of the grain is materially modified. There is a reduction in the amount of starch, some of which is used up by the growing embryo, and an increase in the soluble carbohydrates; also a large quantity of the insoluble nitrogenous matters present in the barley becomes converted into soluble modifications. The figures, quoted by Ling on p. 172, (*J. Inst. Brew.*, 1919, 25, 295) illustrate the differences between some typical barleys and malts.

Well-malted barley ranges in colour from light to dark yellow according to the origin of the barley and the degree of curing. On breaking the malt corn the interior should be pure white, unless the drying has been intentionally carried so far as partially to caramelize the sugar, as, for example, with amber malts. It is customary for the brewer or maltster to form an opinion of a sample of malt from its crispness and flavour. Each corn should break easily between the teeth and the sweet characteristic malty flavour should be quickly developed. If the corns are hard or steely it indicates that the drying has been improperly carried out, too high a temperature having vitrified the corns, or the acrospire has not been sufficiently grown, with the result that the grain is not properly modified. Malt delivered to the brewery should be free from mouldy, broken and damaged corns or culms (dried rootlets).

**Chemical Examination of Malt.**—The brewing value of a sample of malt is dependent chiefly on three factors, namely, the proportion of soluble or extractive matter it will yield to hot water; the character of this extractive matter; and the diastatic activity.

The proportion and composition of the extractive matter are influenced by many conditions, including the temperature of the water used for mashing, the character of the water, the proportion

	English, Norfolk			English, Oxford		Polish		Damascus		Californian	
	Barley	Pale malt	Amber malt	Barley	Malt	Barley	Malt	Barley	Malt	Barley	Malt

Results calculated on the dry samples											
Starch.....	61.56	56.18	55.66	61.05	55.16	58.19	51.34	49.08	54.64	50.28	50.65
Albuminoids, insoluble.....	7.63	5.85	5.58	4.74	6.06	7.53	6.61	5.81	7.21	5.86	5.25
Albuminoids, soluble.....	1.65	3.59	3.35	2.53	4.01	2.93	4.37	4.67	2.52	3.47	3.47
Ash, insoluble.....	1.93	1.65	1.62	1.84	1.99	1.79	1.54	1.49	2.04	1.86	1.80
Ash, soluble.....	0.72	0.80	0.71	0.98	0.66	0.73	0.80	0.84	1.00	0.61	0.66
Fat.....	2.36	1.78	2.07	2.51	1.99	2.47	1.87	2.07	2.02	1.93	1.94
Sugar, invert.....	1.27	4.63	4.30	0.96	3.40	1.43	6.04	6.34	0.56	3.14	3.84
Sugar, cane.....	1.01	5.36	5.68	1.09	6.40	1.27	5.12	6.72	1.90	5.53	7.18
Gums, etc.....	0.95	3.35	3.36	1.48	3.23	1.84	4.06	3.42	1.72	2.83	2.59
Fibre.....	5.63	5.09	5.45	4.99	5.71	5.86	5.41	5.65	6.02	6.48	8.41
1000 corn weight (dry)...	35.46	31.51	31.61	37.98	34.37	37.62	35.17	34.79	41.46	39.11	35.46

The starch figures are probably slightly high and may include the products of hydrolysis of small amounts of pentosans as Ewer's method (*Zeitsch. ges. Brauw.*, 1908, **31**, 250) was used.

employed, the composition of the original malt, and the temperature at which it is dried.

The Council of the Institute of Brewing appointed in 1905 a committee to report upon suitable methods for estimating the extract, moisture, diastatic power, colour, and percentage of ready-formed sugars in malt. A report was issued (*J. Inst. Brew.*, 1906, 12, 1), another report dealing with coloured malts and caramel in 1910 (*ibid.*, 1910, 16, 531) and a further report is now in course of preparation.<sup>1</sup> As these determinations are now usually carried out in all English laboratories associated with the malting or brewing industries they may be regarded, at any rate for the present, as "standard methods."

*Sampling.*—It is obvious that samples sent for analysis should, so far as possible, be fairly representative of bulks, and this requires the more care when the bulks are large, and when the malt contains any appreciable number of hard corns, and further, when there is any marked irregularity in curing.

In the case of deliveries, samples should be drawn from at least one sack in every 10 if the consignment amounts to over 100 sacks, or if the parcel be smaller, then from 10% of the number of sacks. The sample should be withdrawn not from the surface of a sack, but from a depth of at least 6 in.

These bulk samples should be put into a suitable receptacle, thoroughly well mixed up and the requisite number of samples collected in clean, dry, screw-stoppered beer bottles.

If uniform results are to be obtained it is essential that the grists should be uniform; accordingly the committee advised that the standard mill should be set so that the rollers are 0.5 mm. apart.<sup>2</sup> In order to allow for loss in the mill, a quantity of malt, slightly in excess of that required for each determination, should be separately weighed and ground. Finally, the exact amounts of grist subsequently required for the various determinations are weighed out. It is not permissible to grind at the outset sufficient malt for all

<sup>1</sup> This report has now been published and should be consulted by those interested in malt analysis (*J. Inst. Brew.*, 1922, 28, 775-786).

<sup>2</sup> To obviate as far as possible differences in results arising from the gradual alteration in the coarseness of grist yielded by the mill as the result of continuous use, and also to disclose errors introduced by accidental causes it is advisable that the analyst should at the commencement of the season lay in a stock of a good pale malt and that each working day the diastatic activity, extract and moisture of this stock malt should be estimated, no results of other samples being accepted when the results with the stock malt show any appreciable divergence from the normal values. The stock malt may be conveniently stored in dry "Winchester quart" bottles.

the determinations and to weigh the various quantities from this grist.

**Extract.**—50 grm. of ground malt are mashed in a glass or suitable metal beaker (aluminum or brass) of about 500 c.c. capacity with 360 c.c. of distilled water previously heated to such a temperature as will ensure a final temperature of 150° F. (usually 155 to 159° F.). The beaker is covered with a clock glass, and placed in a water-bath, so that its contents are kept at a temperature of 150° F. for 1 hour. The mash is stirred at intervals of about 10 minutes during this time. The mash is then washed into a flask graduated to 515<sup>1</sup> c.c., cooled to 60° F., made up to the mark with distilled water at the same temperature, well shaken and filtered through a large-ribbed paper into a dry vessel, the first 50 c.c. being returned to the funnel. When about 250 c.c. have been collected the sp. gr. of the filtrate is determined at 60° F., compared with water at that temperature.<sup>2</sup> The excess sp. gr. over water (= 1000) multiplied by 3.36 gives the extract in brewers' pounds per standard quarter (336 lb.) of malt.

In dealing with a large number of analyses it is convenient to weigh out 50 grm. of malt (plus one corn) and to mash the ground samples in aluminium or brass beakers.

If preferred, the mashing can be carried out directly in the 515 c.c. measuring flask. In this case the mash should be shaken at intervals of about 10 minutes.

**Colour of Wort.**—For this determination the Lovibond tintometer is employed. The above wort, filtered as bright as possible without the addition of any clarifying agent, should be placed at once in a 1-in. cell, and its tint recorded in colour units of the series "52" glasses. The experiment should not be carried out in direct sunlight, and the light must fall equally on both halves of the white plate so that both fields, *viz.*, the malt-extract field and the stand-

<sup>1</sup> The grains from 50 grm. of malt are supposed to occupy a volume of 15 c.c.

<sup>2</sup> In warm weather it is inconvenient to weigh a sp. gr. bottle containing a liquid at a temperature of 60° F.; 65° or 70° F. are more suitable temperatures. It has, however, been pointed out by G. C. Jones (*J. Inst. Brew.*, 1908, 14, 9) that it is not sufficient to determine the weights of malt extract and water contained by the pyknometer at the same temperature. The results so obtained must be corrected for the difference in the coefficients of expansion. For each degree Fahrenheit that the solution is weighed above 60°, 0.02 is added to the sp. gr. value, while for each degree Fahrenheit below 60°, 0.02 is deducted. This correction is only accurate for malt worts on which the sp. gr. is determined between the temperatures of 50° and 70° F. An example will make it clear. The sp. gr. found for a hot mash-malt extract when weighed at 66° F. = 1027.34.  $6 \times 0.02 = 0.12$ . As the temperature is above 60° F. this amount has to be added. The true sp. gr. of the malt extract is therefore 1027.46. It is, of course, to be understood that the water is always to be weighed at the temperature at which the malt extract is weighed.

ard field, are equally illuminated. To test this, the glasses and the cell should be reversed, and all results rejected when the figures do not agree, whichever side the cell is placed.

J. L. Baker and H. F. E. Hulton (*J. Inst. Brew.*, 1906, **12**, 302; *ibid.*, 1907, **13**, 26) have drawn attention to the discrepancies which arise in reading the colour of worts and beers in the Lovibond tintometer when the position of the instrument is varied relatively to the illumination. It is recommended that the tintometer, in a horizontal position, be directed to a north window,<sup>1</sup> covered with a piece of thin white tissue-paper, and that the opal screen provided with the instrument be discarded. J. W. Lovibond (*ibid.*, 1908, **14**, 2) has devised a standard lamp which it is claimed overcomes the attendant difficulties of daylight as a standard source of illumination.

*Moisture.*—About 5 grm. of finely ground malt are weighed out in a weighing bottle provided with a stopper or in a covered beaker or crystallising dish. The weighing bottle should be about 2 in. in diam. and about 1 in. in depth; the crystallising dish should also have a diam. of about 2 in. The dish and its contents should be kept for 3 hours in a boiling water oven. The stoppers or covers should meanwhile be kept on the top of the oven. At the end of this time the stoppers or covers are replaced and the closed vessels immediately transferred to a desiccator and weighed as soon as cold, the loss in weight being taken as the moisture content and calculated as a percentage on the malt to the nearest first decimal.

Not infrequently considerable differences occur in moisture estimations in malt. The causes of these were investigated in the writer's laboratory, and a paper embodying the results was written by T. J. Ward (*J. Inst. Brew.*, 1919, **25**, 335). The following recommendations were made:

1. Nothing else but malt should be in the drying oven.
2. As the coolest part of the oven is nearest the door, none of the malts should be placed close to the door.
3. The ventilator in the door of the oven must be open and the orifice at the top of the oven kept free. The products of combustion from the burner must be prevented from entering the oven. This may be accomplished by suspending a sheet of metal or asbestos (of the same width as the oven) by means of one edge bent at right

<sup>1</sup> A window facing the south would be of course used in the Southern Hemisphere (Am. Ed.)

angles inserted between the base of the oven and front top of the stand, any remaining crevices being packed with asbestos. The shield should extend at least 15 cm. below the base of the oven.

4. While the malt is being dried in the oven, the oven door is on no account to be opened.

5. The vessels containing the malts must be kept on the floor of the oven.

6. In order to prevent furring, it is advisable to use distilled water for heating the oven.

7. A control determination of moisture-content in a stock sample of malt should be made as above with each batch.

*Diastatic Activity (Lintner Value).*—The measurement of diastatic activity is based on Kjeldahl's law of proportionality (*Compt. rend. des travaux du Laboratoire de Carlsberg*, 1879, 1, 109; *vide* also A. R. Ling, *J. Fed. Inst. Brew.*, 1896, 2, 335; and J. L. Baker and H. F. E. Hulton, *Analyst*, 1921, 46, 90). When working with malt diastase Kjeldahl found that if the production of maltose from starch does not exceed 45% of the starch used, this maltose formed is a linear function of the amount of diastase in the solution.

The method now in general use is that of C. J. Lintner (*Zeitsch. ges. Brauw.*, 1885, 8, 281), which is based on Kjeldahl's work, and involves the use of Lintner's soluble starch (*J. prakt. Chem.*, 1886, 2, 34, 378–394). By this method malt is said to have a diastatic capacity Lintner of 100 when 0.1 c.c. of 5% cold water extract of the malt, if allowed to act for 1 hour at 70° F. upon 10 c.c. of 2% soluble starch solution, produces enough maltose to reduce completely 5 c.c. of Fehling's solution. If 0.2 c.c. were required, the Lintner value will be 50°, and so forth.

*Ling's Method.*—25 grm. of ground malt are extracted with 500 c.c. of distilled water for 3 hours at 70° F.<sup>1</sup> and filtered. The temperature at which the extract is made may be 70 ± 5° F. without influence on the result. The first 100 c.c. of the filtrate is rejected. 3 c.c. of the perfectly bright extract are allowed to act on 100 c.c. of a 2% solution of soluble starch at 70° F. for 1 hour in a 200 c.c. flask. It is essential that these conditions be adhered to.

<sup>1</sup> The water used for this extraction and also for the preparation of the starch solution must be free from ammonium compounds, nitrites and other impurities which may influence diastatic conversion. The water should be redistilled with the addition of a little potassium permanganate and sodium hydroxide until the distillate is pure and neutral to litmus solution. G. C. Jones (*J. Inst. Brew.*, 1908, 14, 12) finds that alizarin paste (1 grm. in 200 c.c.) is a more satisfactory indicator than litmus.

*Preparation of Soluble Starch.*—Purified potato starch is digested with dilute hydrochloric acid, sp. gr. 1.037, at the room temperature (60° to 65° F.) for seven days, stirring the mixture daily; 500 grm. of starch and 1000 c.c. of dilute acid being suitable quantities. The mass is washed very thoroughly by decantation, at first with tap water and later on with distilled water, until the wash water is free from acid. The soluble starch should not be alkaline. The limit of acidity should be that 200 c.c. of a 2 % solution titrated hot against N/10 sodium hydroxide should not require more than 0.2 c.c. of such alkali for neutralisation using alizarin as indicator. It is collected on a filter-paper, placed in a Buchner's funnel, pumped as dry as possible, and then spread out on a new unglazed plate. The starch should be dried at a gentle heat (110° F.) as quickly as possible. When dry, the starch is triturated in a porcelain mortar and rubbed through a fine hair sieve.

*Starch Solution.*—In determining diastatic capacity, the starch must be dissolved in boiling water at the rate of 2 grm. of the starch per 100 c.c. of water; the solution is then cooled to 70° F. for use. It should be mobile (not gelatinous), indicating perfect conversion into soluble starch, should show only a negligible reducing action on Fehling's solution; and it should be neutral to litmus solution.

N/10 alkali (10 c.c.) is then added in order to stop further diastatic action, the liquid cooled to 60° F., made up to 200 c.c. with distilled water at the same temperature, well shaken, and titrated against 5 c.c. portions of Fehling's solution, using ferrous thiocyanate as indicator.

Ferrous thiocyanate is not obtainable commercially and, if it were, would almost certainly be contaminated with ferric salts. It is made as follows:

1 grm. of ferrous ammonium sulphate is dissolved in 10 c.c. of cold water, 1.5 grm. of ammonium thiocyanate is added and, when this has dissolved, 2.5 c.c. of concentrated hydrochloric acid. With the purest available materials, the solution, even when freshly prepared, will usually have a pink tint. This is destroyed by a trace of zinc dust and the solution is filtered. After being kept some time, it re-acquires a red colour, which is again discharged by the use of zinc dust. In this way the indicator may be used for several days, but it finally becomes too insensitive and must be replaced by a freshly prepared solution.

The method of titration is carried out as follows:

5 c.c. of Fehling's solution (see p. 379) are accurately measured into a 150 c.c. boiling flask, and raised to boiling over a small naked Bunsen flame. The converted starch solution is added from a burette, in small quantities at first of about 5 c.c., the mixture being kept rotated and boiled after each addition until reduction of the copper is complete, which is ascertained by rapidly withdrawing a

drop of the liquid by a glass rod and bringing it at once in contact with a drop of the indicator on a porcelain or opal glass slab.

The results are calculated by the following formula:

$$P = \frac{1000}{xy}$$

in which  $P$  equals the diastatic power,  $x$  equals the number of c.c. of malt extract contained in 100 c.c. of the fully diluted starch conversion liquid, and  $y$  equals the number of c.c. of the same liquid required for the reduction of 5 c.c. of Fehling's solution.

The above method (using 3 c.c. of malt extract to 100 c.c. of 2% soluble starch solution) is not accurate for malts having a diastatic capacity exceeding 50 Lintner; in the case of such malts the volume of malt extract must be less, say 2 c.c., or, for malts of the highest diastatic capacity, such as are frequently used by distillers and vinegar makers (*i. e.*, malts of a diastatic power of over 80° Lintner), an even smaller volume of extract must be taken.

An alternative method which is largely employed consists in measuring 10 c.c. of a 2% solution of soluble starch into each one of a series of 8 carefully cleaned test-tubes. The tubes and their content are then placed in a suitable stand and immersed in a water-bath at a temperature of 70° F. As soon as the starch solution has reached this temperature, 0.1 c.c. of the malt extract (prepared as before) is measured into the first of the tubes. The second tube receives 0.2 c.c., the third 0.3 c.c., and so on until the 8 test-tubes contain malt extract in regularly increasing quantities. The tubes are replaced in the stand and immersed in the water bath at 70° F. for exactly 1 hour from the time the malt extract was added to the first tube. To each tube is then added 5 c.c. of Fehling's solution; after shaking, the tubes are heated in a boiling-water bath for 10 minutes and allowed to stand until the cuprous oxide has settled. It will usually be noticed that the liquid of one tube in the series is faintly blue, showing that there was insufficient maltose formed for complete reduction, whilst the succeeding one is yellow due to over-reduction. If, for example, tube 3 was under-reduced as much as tube 4 was over-reduced, the reading would be taken as 0.35 c.c. Intermediate points are judged by inspection. Sometimes the solution in one of the tubes will be neither blue nor yellow, showing that the amount of maltose formed was just enough for complete

reduction. In cases where the reduction lies between the first and second tubes it is advisable to repeat the determination, using a more dilute extract. The results are calculated by the formula:

$$P = \frac{10}{V}$$

in which  $P$  = diastatic power and  $V$  the volume in c.c. of the malt extract judged to be necessary for exact reduction. With malts of a diastatic power not exceeding 100, some workers transfer the contents of any one of those tubes in which the reduction is almost complete to a boiling flask to titrate the unreduced copper with a dilute solution of dextrose (0.2 grm. in 100 c.c.), the exact reducing power of the glucose solution having been determined previously (A. R. Ling, *J. Inst. Brew.*, 1896, 2, 340).

Another procedure has recently been devised by J. L. Baker and H. F. E. Hulton (*Analyst*, 1921, 46, 50) which depends on the oxidation of the maltose formed by alkaline hypoiodite in place of Fehling's solution. The preliminary stages are carried out as in the first method up to the addition of 10 c.c. of N/10 sodium hydroxide solution and the dilution to 200 c.c. Of this liquid, 50 c.c. are placed in a wide-mouthed stoppered vessel, 20 c.c. of N/10 iodine solution added, followed by 30 c.c. of N/10 sodium hydroxide. The whole is then allowed to remain at room temperature for 10 minutes, acidified with 4 c.c. of N/1 sulphuric acid, and the free iodine (excess) titrated with N/20 sodium thiosulphate in the usual manner. The results are calculated by the formula

$$P = \frac{16.7Y}{X}$$

in which  $P$  equals the diastatic power,  $Y$  equals the volume in c.c. of N/10 iodine solution used up, and  $X$  equals the volume in c.c. of malt extract (5%) taken for the conversion.

It is customary to deduct 1.5° from the diastatic power found, to allow for reducing sugars present in the malt extract.

**Cold Water Extract.**—25 grm. of ground malt are digested with 250 c.c. of distilled water containing 20 c.c. of N/10 ammonium hydroxide (*i. e.*, 20 c.c. of N/10 ammonium hydroxide made up to 250 c.c. with distilled water) for 3 hours at 70° F., stirring about three or four times during this period. After filtering, the sp.

gr. of the bright filtrate is taken at 60° F., compared with water at the same temperature. The excess sp. gr. over water (= 1000), corrected for the sp. gr. of the ammonium hydroxide, divided by 3.86 and multiplied by 10 gives the cold water extract per cent. on the malt. The percentage of ready-formed soluble carbohydrates may be approximated by subtracting 4 from the value obtained for the cold water extract per cent.

Considerable importance is still attached by many brewing chemists to the percentage of matter soluble in cold water, as it is claimed to be an indication as to whether a malt has been properly made. If the growth of the sprouted barley is unduly hastened on the malting floor (forcing) more of the starch is converted into sugar and more insoluble nitrogenous matter into soluble matter than if the growth had been slow. Wet loading on the kiln will also occasion an increase in matters soluble in water. In recent years doubts have been expressed as to the value of this determination, for it is by no means proved that a so-called "forced malt" will of necessity produce an unsound beer.

The soluble matters consist of proteins, ash, acid and "ready-formed carbohydrates" (cane-sugar, invert sugar, etc.) and other undifferentiated substances.

*Statements of Results.*—The results, expressed to the nearest first decimal place only, except in the case of *diastatic activity*, which should be recorded only to the nearest integer, are usually set out as follows:

Extract per standard quarter (brewers' pound).

Moisture, per cent.

Diastatic power (Lintner).

Colour (10% wort, 1-in. cell, "52" series Lovibond).

Cold water extract, per cent.

In addition to the estimations described above, the following afford information of a useful character.

*Modification.*—Malts differ considerably in the extent to which modification has taken place. If the growth has been insufficient on the floor the finished malts will have steely ends, and these will not yield the full extract when mashed, as the starch will not be amenable to the action of diastase. Modification may be conveniently measured by mashing a fine and coarse grind of the same malt

under the conditions previously given. In a well-made malt the extracts of a fine and coarse grind will be practically the same, in steely malts the differences may be considerable (3 to 4 lb. per quarter).

*The "Saccharification" Test.*—This test has been devised to measure the time required for the complete saccharification of a malt mash. 10 grm. of the ground malt are mixed with 100 c.c. of water at 154° F. and kept at 151° F. in a suitable bath, the mash being stirred occasionally. In 15 minutes about 5 c.c. of the mash are withdrawn, filtered and the cooled filtrate tested for the presence of starch by iodine. If starch is found the test is repeated at intervals of 5 minutes until the iodine reaction is no longer observed. The time taken for the complete saccharification is then noted (A. J. Brown, *Laboratory Studies for Brewing Students*, p. 62).

*Dry Grains.*—The concentration of the wort (wort solids) in the extract estimation is arrived at by dividing the excess gravity above 1000 by 4. As the proportion of malt used to water was as 10:100, the dry extract multiplied by 10 represents the dry extract in 100 grm. of malt. The percentage of dry extract subtracted from 100 gives the percentage of dry grains. The result is, of course, corrected for the moisture in the malt.

### Physical Examination

*Growth.*—100 or preferably 200 malt corns are counted and sorted into the following six groups according to the development of the acrospire up the back of the corn, 0 to  $\frac{1}{4}$ ;  $\frac{1}{4}$  to  $\frac{1}{2}$ ;  $\frac{1}{2}$  to  $\frac{3}{4}$ ;  $\frac{3}{4}$  to 1; overgrown, dead and damaged corns. In well-made malts 80 to 100% of corn are from three-fourths to fully grown. When dealing with malts made from foreign barleys such as Californian, Smyrna, American, etc., it is usually necessary to remove the skin of the corn or the length of the acrospire cannot properly be judged.

*The "Sinker" Test.*—500 corns (about 17 grm.) are counted and stirred into a beaker containing cold water. The corns which float are removed. Some corns will lie flat on the bottom of the beaker, and when these are examined they will be found to be either ungerminated barley, very steely, or vitreous corns; other corns may rest on one end and these will probably be steely-tipped. In the writer's experience it is preferable to use brine of sp. gr. 1.120

in place of water. In this solution only barley sinks, the poorly the well-modified corns float. It is a convenient way of estimating the quantity of ungerminated barley in a malt (Idlers).

Malt should be free from impurities, such as stones, (which may cause explosions during the grinding) dirt, or foreign seeds. Serious arsenical contamination now rarely occurs. If present in quantity (a safe limit is  $\frac{1}{300}$  gr. of arsenious oxide per pound) it may usually be traced to carelessness on the part of the maltster, such as mixing gas coke with the anthracite. Another and rather common source of arsenical contamination arises from cleaning and brushing out the kiln and using it again before the dust has had time to deposit. The dust in a malt kiln contains arsenic derived from the fuel used. For the estimation of arsenic in malt and beer see page 195.

#### SPECIMEN ANALYSES OF MALTS

	English mild ale malt	English amber malt	English pale malt	Cali- formian malt	Smyrna malt
Extract (pounds per 336 lb.)	96	94	97	88	86
Colour, degrees	7	20	4	3	3
Moisture, per cent	2.5	2.0	2.4	2.0	2.2
Diastatic power (Lintner)	25	14	35	34	35
Cold water extract, per cent	20	21	20	17	17
Grains, per cent	27	27	27	32	36
Growth:					
Full grown, per cent	2	..	5	10	6
Three-fourths grown, per cent	75	80	79	70	73
One-half grown, per cent	20	20	14	15	16
Ungerminated corns, per cent	1	..	1	2	3
Damaged corns, per cent	2	..	1	3	2
Modification	Good	Good	Very good	Good	Good

#### Malt Worts

*Total Solid Matter.*—The sp. gr. of a malt wort is ascertained in the laboratory by means of a sp. gr. bottle, and this figure minus 1000 (water = 1000) divided by 4 gives the number of grams of solid matter dry extract) contained in 100 c.c. of the wort.

For the purposes of the brewer the sp. gr. of the wort may be ascertained by the hydrometer, various modifications of which have been devised for this purpose.

Bates' brewers' saccharometer is an instrument the indications of which are expressed in "pounds per barrel," and these may be

translated into absolute sp. gr. by dividing the number of "saccharometer pounds" by 0.36 (or multiplying by 2.778) and adding 1000. A barrel (= 36 gallons) of water weighs 360 lb.; a beer-wort, a barrel of which weighs 380 lb. (= 360 + 20), is said to have a "saccharometer gravity of 20 lb. per barrel." The real sp. gr. of such wort would be 1055.5; — for  $360 : 380 = 1000 : 1055.5$ ; and it would contain 13.8 grm. of solid extract per 100 c.c. or 50.1 lb. per barrel of 36 gallons. Similarly, a wort of 1055 sp. gr., which is the standard strength of beer wort on which the duty of £5 per barrel is levied, has a saccharometer gravity of 20.52 lb. per barrel; for  $1055 - 1000 = 55$ ; and  $55 \times 0.36 = 19.80$ .

Corrections of sp. gr. of beer worts for temperature can be made as described on page 174.

The method of ascertaining the original gravity of malt or beer worts which have undergone fermentation is described on page 203.

The solid matter of malt worts consists of a mixture of dextrins, sugars, nitrogenous matters and ash constituents. The work of O'Sullivan, Brown and Morris, Lintner, Ling, Baker and Hulton and many others has shown how very complicated are the products formed by the action of diastase on starch.

In the mash tun, maltose, a series of dextrins differing in molecular weights and complexity (the so-called malto-dextrins), probably dextrose and the pre-existing carbohydrates in the malt are present. To follow the nature of the conversion in the mash tun by fully analysing the wort involves an amount of work which, in the opinion of the writer, is not warranted by the results obtained.<sup>1</sup>

Some workers claim that useful data for control purposes are furnished by the specific rotatory power and cupric reducing power of the wort and from these the percentage of apparent maltose and apparent dextrin on the wort solids may be calculated.

*Estimation of "Apparent Maltose and Dextrin."*—The wort is boiled to throw out any coagulable proteins, filtered and the sp. gr. determined at 15.5°/15.5°. This figure minus 1000 and divided by 4 will give the grams of wort solids per 100 c.c.

*Specific Rotatory Power.*—A wort light in colour may be read directly in a 100 or 200 mm. tube. If the wort should be dark it may be clarified with basic lead acetate or alumina cream as in the case

<sup>1</sup> Moritz and Morris describe an elaborate scheme for the analysis of worts in their *Text-book of the Science of Brewing*. (E. and F. N. Spon.)

of raw sugars.<sup>1</sup> Black beer worts require a special treatment, as basic lead acetate will not remove all the colour. The late J. Heron suggested treating the wort with bleaching powder, but highly caramelised worts are not always sufficiently decolourised by this process for reading in a polarimeter. The writer has found that any black wort may be decolourised with phosphotungstic acid. The reagent is prepared by dissolving phosphotungstic acid in water (4%) and adding 20% sulphuric until there is a slight turbidity. To 25 c.c. of the original black wort 4 c.c. of phosphotungstic acid are added and 10 c.c. of 20% sulphuric acid. The contents of the flask are made up to 100 c.c., filtered and read. An average value for the  $(a)_D$  expressed on solids of an all-malt mash tun wort is  $+110^\circ$  to  $+115^\circ$ .

*Reducing Power.*—For this determination the conditions advised by Brown, Morris and Millar are very commonly employed (*J. Chem. Soc.*, 1897, 71, 72). However, for rapid and accurate work, the volumetric process as modified by Ling and Rendle (*Analyst*, 1908, 33, 167, see also page 176) may be recommended, and if carried out under standard conditions the results are probably quite as accurate as the gravimetric method (Ling and Jones, *ibid.*, 1908, 33, 167).

For the purpose of the calculation the specific rotatory power of maltose is taken as 138 and dextrin as 200. All reducing sugar present is supposed to be maltose. The grams per 100 c.c. of maltose  $\times 1.38$  = rotation due to maltose. The total reading minus that due to the "apparent" maltose divided by 2.00 = dextrin in grams per 100 c.c.

The percentage of "apparent maltose" in mash tun worts calculated on the wort solids varies between 70 and 80, the "apparent dextrin" between 4 and 10.

### Roasted Barley and Malt

These materials are used principally in the brewing of stouts; small quantities are sometimes added to a pale malt grist for the purpose of adjusting the colour of a beer. For technical control purposes it is customary to determine extract, colour and moisture.

<sup>1</sup> This is the conventional way of clearing sugar solution but Davis and Daish (*J. Agric. Science*, 1914, 6, 152) have shown that although basic lead acetate does not of itself precipitate dextrin, when dextrin is present in solutions in which a precipitate is produced, it is carried down with this precipitate and is thus lost to the analysis.

The high temperature employed in the making of roasted barley so modifies the character of the starch in the grain as to make it soluble in hot water. It is therefore unnecessary for the determination of the extract to make a mash with a pale malt or with cold water malt extract as in the case of brown malts or flakes.

*Extract.*—A little over 50 grm. is finely ground in a coffee mill, and exactly 50 grm. of the ground product are mixed with about 350 c.c. of boiling distilled water and the mixture kept in a boiling water-bath for an hour. It is then cooled, made up to 515 c.c., filtered and its sp. gr. at 60°/60° taken. The excess gravity (over water = 1000) multiplied by 3.36 gives the extract in brewers' pounds per standard quarter of 336 lb. Black barleys and malts are not commonly purchased on the basis of the standard quarter of 336 lb., various weights and measures being employed, but the Malt Analysis Committee of the Institute of Brewing recommends the above uniform method of stating analytical results, leaving subsequent calculation to whom it may concern. The extract varies according to the quality of the barley roasted. A usual figure is 84 to 90 lb. per 336 lb.

*Colour.*—20 c.c. of the above extract are diluted to 1000 c.c. (0.2% solution) and the colour read in 1-in. cell, using Lovibond's tintometer and glasses of "Series 52." A roasted barley should have a colour of at least 25°. Roasted malts are frequently slightly higher.

*Moisture* is estimated in the same manner as in a malt.

Roasted barley is now largely taking the place of roasted malt, the latter being used mostly in the brewing of export stouts. Since roasted malt is more expensive than roasted barley, it is necessary to see that the former when ordered is delivered. Usually this can be done by observing if the acrospire shows any sign of development. The lower nitrogen content of roasted malt as compared with barley has been proposed as a means of differentiation, but the wisdom of estimating nitrogen in bodies which have been submitted to such high temperatures as to be charred is doubtful.

### Brown and Crystal Malts

Under this heading may be considered a number of semi-roasted malts which are used in brewing for flavouring and colouring purposes. Usually extract, colour and moisture are estimated. The methods

of analysis commonly used are those recommended by the Institute of Brewing (*J. Inst. Brew.*, 1922, 28, 775).

*Extract*.—50 grm. are ground in the standard mill, so set that there is a distance of 0.5 mm. between the rollers, as determined by a feeler gauge. The grist is mashed with 300 c.c. of distilled water at 158° F. and 100 c.c. of cold-water malt extract<sup>1</sup> previously heated to 150° F. The mixture is kept for an hour at 150° F., then cooled to 60° F., made up to 515 c.c., filtered and the sp. gr. of the filtrate taken at 60° F. Simultaneously, 100 c.c. of the cold-water malt extract, mixed with 300 c.c. water, are digested for an hour at 150° F., then cooled to 60° F., made up to 500 c.c., filtered and the sp. gr. of the filtrate taken. The difference between the sp. gr. (water = 1000) of this filtrate and that obtained in the experiment with brown or crystal malt, multiplied by 3.36, gives the extract of the malt in brewers' pounds per standard quarter of 336 lb. Brown and crystal malts are not commonly purchased on the basis of the standard quarter, various weights and measures being employed, but the above uniform method of stating results is recommended.

*Colour*.—20 c.c. of the above extract, which must be brilliant, are diluted to 100 c.c. (2% solution) and the colour read in a 1-in. cell, using a Lovibond tintometer and glasses of "Series 52."

*Moisture* is estimated as in ordinary malt.

### Malt Substitutes

In recent years many of these preparations have been placed on the market. Most of them are derived from maize or rice. The starch in the grain is rendered amenable to diastatic action by being submitted to a torrefaction process; that is, the combined action of moisture and heat. Since these substitutes are used solely for the extract they yield in the mash tun, an estimation of the matter capable of being dissolved by malt extract is of importance.

This extract may be measured by mashing a mixture either of 25 grm. of malt and 25 grm. of maize or 16.7 grm. of maize and 33.3 grm. of malt making up to 515 c.c. under the same conditions as malt (see above), the resulting extract due to the flakes being multiplied by 2 or 3, as the case may be. It is preferable to mash equal quanti-

<sup>1</sup> The cold-water malt extract is made by digesting malt of diastatic power 30° to 40° Lintner with three times its weight of distilled water for 1 hour at 60° to 70° F., and then filtering.

ties of flakes and malt, as any error in analysis is multiplied by 2 instead of 3. J. L. Baker (*Brewers' J.*, 1905, 41, 186) has pointed out that the extract obtained from flakes differs with the diastatic capacity of the malt employed. If deliveries of flakes are controlled by analysis, the same malt should be used by the chemist of the buyer and seller. In this way only is it possible to obtain comparable results. A malt of a diastatic power not exceeding 30° should be used. Briant (*J. Inst. Brew.*, 1905, 11, 395) suggests mashing the flakes with an extract prepared by digesting a pale malt of a diastatic power of 30° to 40° Lintner with three times its weight of cold water for 90 minutes. 20 grm. of the flakes are placed in a beaker, 120 c.c. of water added and the temperature raised to 160° F., carefully stirring during the time. 50 c.c. of the cold-water malt extract are run slowly in, the whole mixed and allowed to stand at a temperature of 150° F. for 2 hours. The mash is transferred to a 200 c.c. flask, cooled to 60° F., and made up to bulk, filtered and the sp. gr. taken. This, less the gravity due to the added malt extract (which is treated in a similar manner) represents the gravity due to the flakes. The excess gravity multiplied by the factor 3.32 will give the extract yielded by 336 lb. of the flakes. (The volume occupied by the grains from 20 grm. of flaked maize is on the average 2.5 c.c., and the factor has been calculated so as to allow for this.) The method gives satisfactory results.

In judging the suitability of flakes for brewing purposes the amount of oil should be noted, as this constituent may impart an unpleasant flavour to the finished beer. The oil may be estimated by extracting 5 grm. of the finely powdered flakes in a continuous extractor with ether for 3 hours. The ether is evaporated and the residual oil dried in a boiling water-bath for 1 hour, cooled in a desiccator and weighed. Carefully prepared flakes contain about 1% of oil; if more than 2% is present they are not suited for brewing.

Moisture is estimated as in malt. An average figure is 8 to 10%.

The extract is usually 95 to 100 lb. per quarter of 336 lb.

### Grits and Raw Grain

These are used as a source of extract in some breweries. They are treated in a converter to gelatinise the starch, cooled to a convenient temperature and mashed with malt. Such materials may be analysed

by heating with water preferably under pressure, and treating the starch paste so produced with malt or malt infusion of known extract. Grits should also be examined for oil and moisture.

### Malt Extract

Malt extract occurs as a light yellow or amber-coloured, thick, viscid liquid, having a faint, pleasant, characteristic odour, a sweet mucilaginous taste and a distinctly acid reaction. It is soluble in all proportions of water, but the solution gives a precipitate with strong alcohol. Its diastatic activity is destroyed at temperatures above 65°.

Dry malt extracts are mostly used for pharmaceutical purposes or as a constituent of infants' and invalids' food.

The medicinal value of malt extract depends upon the proportion of total solid nutritive carbohydrates it contains and upon its diastatic action. Many of the extracts on the market contain little or no diastase, the enzyme having been destroyed during evaporation.

Malt extract now commands considerable attention in breweries as a yeast food, especially in cases where there are indications of yeast weakness. It can be used with advantage in the mash tun if the percentage of malt substitutes, such as flaked maize, is high or if the malts are old and slack. For such purposes malt extract should have a diastatic power of 80 to 100° Lintner. Some brewers add the extract to the copper. Malt extract also finds some favour as a priming in cases where the racking gravity of the beer is high.

Various proprietary malt extracts<sup>1</sup> are largely used as a solvent for starch in the cotton and laundry industries. Their value depends entirely on their enzymic activity and particularly their liquifying power towards starch paste. The analytical examination of malt extracts intended for these purposes has been the subject of considerable enquiry. Those interested should read papers by May (*J. Soc. Dyers and Col.*, 1911, 27, 88), Hamburg (*ibid.*, 1912, 28, 7) and Dreaper (*ibid.*, 1913, 29, 157; 1914, 30, 255).

The following analyses of commercial preparations were made by A. R. Ling (*Analyst*, 1904, 29, 244):

<sup>1</sup> With malt extracts may be classed extracts of *aspergillus* such as Takadiastase. (Am. Ed.)

	I	II	III	IV	V	VI
Sp. gr. 15.5°/15.5°	1395.70	1395.12			1408.43	1377.82
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Maltose (apparent).....	31.1	30.9	24.8	27.4	34.2	25.2
Dextrose.....	17.2	18.2	22.0	19.1	12.5	20.0
Dextrin (apparent).....	9.8	8.6	10.0	9.8	9.9	6.7
Unfermentable matter (expressed as dextrin)	4.5	3.5	8.9	5.8	.....	.....
Ash.....	1.45	1.49	1.58	1.64	1.34	1.64
Water.....	24.30	24.07	27.36	24.84	24.38	29.52
Diastatic power (Lintner)	30.8	27.2	32.3	25.6	39.2	46.5
Specific rotatory power [α] <sub>D</sub> , degrees.....	91.8	90.5	84.2	86.8	94.5	81.1

According to W. J. Sykes and C. A. Mitchell (*Analyst*, 1901, **26**, 230), the total solids range between 75 and 82%; phosphoric acid (P<sub>2</sub>O<sub>5</sub>), between 0.5 and 1.15, and total nitrogen, between 0.4 and 2.25. The presence of dextrose in authentic samples of malt extract, as pointed out by Ling (*loc. cit.*), is of importance and should be borne in mind when adulteration with "glucose syrup" is suspected.

The method for the estimation of total solids is the same as that used in the analysis of malt wort. Owing to the presence of dextrose, cane and invert sugar, it is not possible to estimate all the sugars present in terms of apparent maltose and dextrin by the copper method in conjunction with the polarimeter alone. Ling (*loc. cit.*) determines the dextrose as glucosazone (see under sugar), the maltose being calculated from the reducing power less that due to the amount of glucose found, whilst the dextrin is calculated from the rotatory power after deducting that due to the dextrose and maltose.

In the course of the last few years the writer has had occasion to examine many malt extracts intended for brewing purposes. The following figures are typical of the preparations now on the market:

	American malt extract	English malt extract	English malt extract	Dry extract	Malt extract priming
Sp. gr. of 10% solution	1032.46	1030.4	1030.7	1034.2	1030.98
Total solid matter, per cent	83.2	77.0	78.6	85.6	79.4
Water	16.8	22.1	21.4	5.6	20.6
Unfermentable matter			45.0	.....	47.0
Sucrose, per cent	13.3	6.0	5.0	3.3	Trace
Colour of 10% solution	15.0	6.0	62.0	.....	.....
1-in. cell Lovibond tintometer	1.06	1.12	1.28	2.23	0.42
Mineral matter, per cent	6.1	6.2	4.05	.....	.....
Protein.....	+124.0	+92.1	+109.4	+120.0	+139.6
Rotatory power [α] <sub>D</sub> .....	61.7	94.6	83.5	70.0	67.0
Reducing power as maltose	Nil	97.0	3.0	.....	Nil
Diastatic power (Lintner)	71.8	68.1	68.7	70.7	60.4
Brewers' extract, pounds per 2 cwt					

### Caramel

Caramels are prepared by heating sucrose or glucose with small quantities of an alkali such as ammonia or its carbonate, sodium carbonate or caustic soda. They are used in the fermentation industries as colouring agents and may be added before or after fermentation.

*Extract.*—10 grm. are dissolved in distilled water, made up to 100 c.c., filtered and the sp. gr. of the filtrate determined at 60°/60° F. The excess gravity (water = 1000) multiplied by 2.24 gives the extract in brewers' pounds per 2 cwt. This figure is usually 63 to 68 lb. per 224 lb.

*Colour.*—10 c.c. of the above solution are diluted to 1000 c.c., and the colour read in a 1-in. cell (0.1% solution), using a Lovibond tintometer and glasses of "Series 52." Good brewers' caramels have a colour of 30° to 40°.

*Ash.*—3 grm. are treated with 2 c.c. of sulphuric acid in a tared dish, which is then heated over a flame until intumescence is complete, when it is transferred to a muffle and the contents are incinerated at a low red heat. It is usual to deduct 10% from the weight of the ash found, as in the analysis of sugars, though the mineral constituents of caramel are so frequently mainly sulphates that no correction, or at most a very small one, is really needed.

*Iron* is nearly always present in detectable amount, and, since brewers object to more than a minute trace of iron in their materials, it is usual to estimate the iron—which should not exceed 0.03% Fe (calculated on sample)—in the ash by dissolving the latter in hydrochloric acid, adding potassium thiocyanate and comparing the colour produced with standards containing known amounts of ferric iron and the same concentration of acid and thiocyanate (Thomson: *J. Chem. Soc. Trans.*, 1885, 493).

Caramel for use in brewing should throw down no sediment when mixed with beer. The test is usually continued for 24 hours. If prolonged beyond this period, as is sometimes worth while, some of the same beer without caramel should be set up alongside, lest a sediment due to the beer itself be attributed to the caramel. The loss on fermentation, which should not exceed 5%, may be determined by adding a solution of the caramel to some malt wort together with

yeast and reading the colour in a tintometer before and after fermentation.

### Invert Sugar

Invert sugar, made by hydrolysing raw cane sugars with acid, is an important brewing material and is subjected in brewing laboratories to a much more exhaustive scheme of analysis than is to be found under this heading in the section on "Sugars."

Invert sugar made from refined sugar lacks the lusciousness and other characteristics desirable in a brewing sugar, so that raw cane sugars are generally used. In addition to invert sugar, uninverted sucrose and water, commercial invert contains from 0.2 to 0.7% of proteins, from 3 to 6% of unfermentable organic matter and from 1 to 3.5% of mineral matter, the latter being partly derived from the raw material and partly introduced as calcium carbonate to neutralise the acid used in effecting hydrolysis. Sulphuric acid is generally employed as hydrolyst because the comparative insolubility of calcium sulphate makes it possible to eliminate most of the mineral matter introduced for the purpose of neutralization.

Raw beet sugar could not be used for the production of brewers' invert, on account of the objectionable flavour of the secondary constituents. No such objection would attach to the use of highly refined beet sugar, but highly refined sugars are not used for the reasons already stated. Occasionally invert sugar has been made from a mixture of raw cane sugar and high-grade raw beet sugars (first runnings) and the origin of such invert sugar is not readily detected by the palate or nose. It is, however, desirable to exclude it from the brewery, and this can usually be done by limiting the permissible percentage of proteins, which is higher in beet than in cane products. Brewers' invert is supplied in three grades, and it is reasonable to require them to contain less than the following percentages of proteins: No. 1, 0.3%; No. 2, 0.5%; and No. 3, 0.75%. A good No. 3 will comply with the standard here set up for No. 1, so that the above limits are not unduly stringent.<sup>1</sup>

The analysis of commercial invert includes the following determinations: dextrose, lævulose, sucrose, proteins, ash, unfermentable matter and water. The difference between the sum of these and 100 is returned as "other organic matter." The brewers' extract per 2

<sup>1</sup> CP. Ling, *J. Inst. Brew.*, 1914, 20, 185.

cwt. is also an important figure, always determined, and the colour may be.

*Ash.*—This is determined as in caramel (*q. v.*) and it is customary to deduct 10% from the weight of the sulphated ash as with other sugars, although the correction is no doubt too large for a product inverted with sulphuric acid.

*Water and Brewers' Extract.*—25 grm. are dissolved in distilled water, the mixture made up to 250 c.c., filtered, and its sp. gr. determined at 60°/60°. The excess gravity (water = 1000) multiplied by 2.24 gives the extract in brewers' pounds per 2 cwt.

*Water.*—A 10% solution of pure invert sugar has a sp. gr. of 1038.7 and, in solutions of approximately this concentration, the excess sp. gr. is very nearly proportional to the concentration. The composition of a solution of pure invert sugar can therefore be calculated by dividing the excess gravity by the "solution factor" 3.87. The solution factor of uninverted sucrose is 3.86, and that of the other organic solids of commercial invert probably not very different, whilst their amount is small. The solution factor of the ash constituents, on the other hand, approximates to 8 and in brewery laboratories is usually assumed to be double that of the organic solids. The percentage of total solids in the invert is therefore arrived at by dividing the excess gravity of the 10% solution by 0.387 and subtracting the percentage of ash. The difference between the result so found and 100 is the percentage of water in the sample.

*Proteins.*—Nitrogen is estimated by Kjeldahl's method and the result multiplied by 6.25. If unduly high, a separate determination of nitrate nitrogen should be made before condemning the sample on its high content of "albuminoids."

*Invert Sugar and Sucrose.*—These can be estimated by a method worked out by Morris (*J. Inst. Brew.*, 1898, 4, 162) who first devised a means of overcoming the interference of optically active and reducing non-sugars. The following method, based on that of Morris, is easier of execution and has been found by Ling (its originator) and G. C. Jones to be capable of a somewhat higher degree of accuracy. It depends on the volumetric estimation of the reducing sugars and the use of the table constructed by Ling and Jones (*Analyst*, 1908, 33, 160. (See under Sugars, page 419.)) The table was constructed from experiments made with Fehling's solution of which 10 c.c. required 25.65 c.c. of 0.2% pure invert

sugar. If another worker finds that 10 c.c. of his Fehling's solution requires under his conditions only 25 c.c. of 0.2% invert sugar, he will need to reduce the numbers in columns *D*, *L*, *I* and *M* and increase those in columns *D'*, *L'*, *I'* and *M'* proportionately. The maltose columns are for use in the analysis of commercial glucose (*vide supra*).

To make clear the calculations involved, the following description of the method is accompanied by a worked example.

The rotation of the 10% solution, prepared for the estimation of water and brewers' extract, is observed at 20° in a 200 mm. tube in a Ventzke-Scheibler half-shadow polarimeter. 10 c.c. of this solution are diluted to 500 c.c. and the reducing power of this 0.2% solution determined by titration with 10 c.c. portions of Fehling's solution, using ferrous thiocyanate as indicator see p. 420.

In a particular case, a commercial invert sugar gave a reading in 10% solution in a 200 mm. tube of -4.7 divisions and 10 c.c. of Fehling's solution required 36.45 c.c. of 0.2% solution for reduction.

10 c.c. of the 10% solution are diluted to about 150 c.c. and boiled for 1 minute with 30 c.c. of N/2 hydrochloric acid to invert the small amount of sucrose always present in commercial invert sugar. The mixture is cooled, neutralised with 30 c.c. of N/2 sodium hydroxide, diluted to 500 c.c. and titrated against Fehling's solution.

In the case cited, 10 c.c. of Fehling's solution required 35.95 c.c. of the completely inverted 0.2% solution. From this result and the reducing power of the 0.2% solution before inversion, the percentage of sucrose is calculated. Reference to column *I* of the table shows that the reducing power of the inverted solution corresponds to the presence of 0.1460% of apparent invert sugar, that of the uninverted solution to 0.1441%. The difference, 0.0019, is the measure, in terms of invert sugar, of the sucrose in 0.2 gram. of the sample, which therefore contained  $(0.0019 \times 0.95 \times 500 =)$  0.9% sucrose.

25 gram. of the original sample are dissolved in about 200 c.c. of water, to which about 5 c.c. of yeast water and 3 gram. of washed, pressed yeast are added, and the mixture is allowed to ferment at about 70° F. for 3 to 4 days. A little alumina cream is then added, the mixture made up to 250 c.c., filtered and its rotation and reducing power determined.

In the case cited, the fermented 10% solution gave a reading in a 200 mm. tube of  $-0.1$  division, and 40 c.c. was required to reduce 10 c.c. of Fehling's solution. Opposite 40 c.c. in column *I* of the table is the number 0.1319, the reducing power in terms of invert of the unfermentable matter in 10% solution. In 0.2% solution, therefore, the unfermentable matter would raise the apparent content of invert sugar 0.0026%. The table also shows that in a concentration such that 10 c.c. Fehling's solution require 36 to 37 c.c., a difference of 0.0037% of invert sugar makes a difference of 1 c.c. in the burette reading. Unfermentable reducing substances equivalent to 0.0026% invert would therefore reduce it 0.70 c.c. 10 c.c. of Fehling's solution would therefore require  $(36.45 + 0.70 =)$  37.15 c.c. of the 0.2% solution, if this were free from unfermentable reducing substances, or 1 grm. of the sample contains dextrose and lævulose equivalent to  $10 \div (37.15 \times 0.002) = 10 \div 0.0743 =$  134.6 c.c. Fehling's solution.

In concentrations such as that in which the first reduction experiment was made—namely, where 10 c.c. Fehling's solution require 36.45 c.c. of sugar solution—1 grm. dextrose = 196.6 c.c. Fehling's solution and 1 grm. lævulose = 183.8 c.c. Fehling's solution. If the percentage of dextrose in the sample be represented by *D* and the percentage of lævulose by *L*, it follows that

$$1.966D + 1.838L = 134.6 \quad (1)$$

The sample was found to contain 0.9% sucrose. A 0.9% solution of sucrose gives a reading of  $(3.85 \times 0.09 =)$   $+0.3$  division when read in a 200 mm. tube in a Ventzke-Scheibler polarimeter. The actual reading ( $-4.7$ ) must therefore be corrected for this amount, as well as for the reading of the unfermentable residue ( $-0.1$ ), in order to arrive at the reading due to dextrose and lævulose alone,  $-4.7 - 0.3 - (-0.1) = -4.9$  divisions. Since 1% solutions of dextrose or lævulose give readings of 3.05 and  $-5.32$  divisions respectively, it follows that

$$0.305D - 0.532L = -4.9 \quad (2)$$

From equations (1) and (2),  $D = 39.0$  and  $L = 31.5\%$ .

### Starch Sugars

Glucose chips contain in addition to dextrose, maltose (nil to 12%), dextrin (up to 14%), water (usually 13 to 14%), small amounts

of mineral matter (0.5 to 2%) and still smaller amounts of proteins (0.2 to 0.6%). Glucose syrup or dextrin-maltose usually contains rather less than 25% of dextrose, rather more maltose,<sup>1</sup> over 30% of dextrin, about 18% of water and traces of mineral matter and proteins.

As regards ash, proteins, water and brewers' extract, these sugars are analysed like commercial invert sugar (*q. v.*).

Dextrose and maltose are estimated in a manner similar to that employed for the estimation of dextrose and lævulose in invert sugar, except that no inversion experiment is necessary and that the equations to be used are:

$$aD + 1.225M = F \quad (1)$$

$$0.305D + 0.798M = R \quad (2)$$

where  $a$  is a coefficient found by reference to the table on page 399 after experiment, as in the analysis of invert sugar,  $F$  is the number of c.c. of Fehling's solution corresponding to 1 grm. of the starch sugar, duly corrected for the reducing power of the unfermentable residue, and  $R$  is the rotation in divisions Ventzke (200 mm. tube) or a 10% solution of the sugar, duly corrected for the rotatory power of the unfermentable residue. The coefficient of  $M$  in equation (1) is a constant, independent of the concentration.

The difference between 100 and the sum of the percentages of dextrose, maltose, water, ash and proteins may be, and usually is, returned as "dextrinous carbohydrates and other organic matter." The percentage of actual dextrin is approximately given by dividing the rotation (Ventzke, 200 mm. tube) of the fermented 10% solution by 1.166.

*Estimation of Arsenic in Beer and Brewing Materials.*—The estimation of this impurity is somewhat out of place in a volume devoted to "organic analysis," but since the alarm occasioned by the arsenical contamination of beer in 1900 to 1901, the testing of brewing materials and beers for arsenic has become a matter of routine. Although excellent methods have been devised for this, the Marsh-Berzelius process, with recent improvements, is, in the opinion of the writer, the simplest.<sup>2</sup>

<sup>1</sup> Kluyver (*Biochemisch Scheikerbepalingen*, Leiden, 1914) states that a series of analyses, made by discriminating yeasts, of commercial glucose syrups showed the presence of 15 to 20% of maltose in the products he examined.

<sup>2</sup> This method is similar to that recommended by a joint committee of the Society of Public Analysts and the Society of Chemical Industry (*J. Soc. Chem. Ind.*, 1902, 21, 94).

The reports, minutes of evidence and appendices on the Royal Commission of Arsenical Poisoning, 1902, may be regarded as a text-book on this subject. The standard electrolytic method is described at length in Appendix 21, page 208 of the report.

### Preparation of Materials

**Zinc.**—Since arsenic is frequently present in zinc it is necessary to ascertain if the latter is free from this impurity and also if it is sufficiently sensitive, or in other words, if it produces a normal arsenic deposit from a solution containing a known amount of arsenious oxide. Before use the zinc must be granulated. The outer surface of the ingot is cleaned by scraping, then treated with arsenic-free hydrochloric acid and well washed with water. The zinc is melted in a porcelain crucible and when just molten it is poured from a height of about 4 ft. into cold water. Chapman (*Analyst*, 1907, **32**, 247) has proposed the addition of a cadmium salt to increase the sensitiveness of the zinc. Many dealers now supply granulated zinc, sufficiently pure to be used in the estimation. Each quantity purchased, however, should be tested for arsenic, and for "sensitiveness."

**Hydrochloric Acid.**—This acid unless specially purified frequently contains sufficient arsenic to render its application for the test useless. The method devised by Thorne (*Proc. Chem. Soc.*, 1902, **18**, 118) works well in practice. Ordinary strong hydrochloric is diluted with water placed in a large retort. Through the stoppered opening is introduced a glass rod carrying on the end a piece of very fine copper gauze. The contents of the retort are gently boiled for an hour, the glass rod and copper gauze removed, a small piece of fresh gauze added and the acid distilled. The first 100 c.c. of the distillate should be rejected. The purified acid used in the test should have a sp. gr. 1.10.

Another and more convenient method is to dilute 1500 c.c. of the "pure" strong acid of the dealer to 5000 c.c. The resulting sp. gr. will be about 1.050°. 5 grm. of granulated zinc free from arsenic are added and allowed to dissolve. When dissolution is complete 40 c.c. of stannous chloride solution are run in. The latter solution is prepared by boiling gently for 1 hour, 25 grm. crystallised stannous chloride, 17 c.c. strong hydrochloric acid, 200

c.c. water and 1 gram. of granulated tin. The solution is cooled, diluted to 200 c.c. and with a few grains of granulated tin placed in the containing bottle.

*Apparatus.*—The flask (capacity 175 c.c.) is fitted with a ground-glass stopper through which passes the stem of a funnel furnished with a stop-cock. The stopper also carries the exit tube on which is a bulb bent twice at right angles and connected with the tube containing calcium chloride and a plug of lead acetate wool or paper. The hard glass tube on which the arsenic is to be deposited is made of Jena tubing (external diameter 5 mm.), the drawn-out portion having an external diameter of 2 mm., at a distance of 15 mm. from

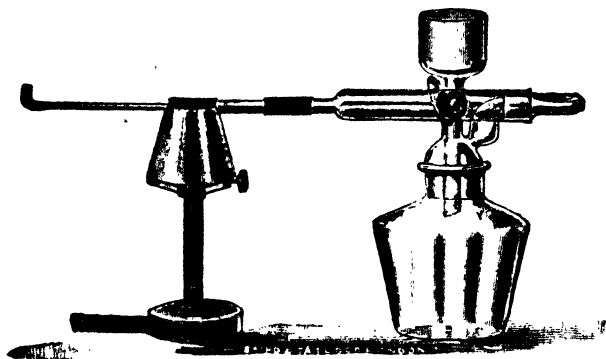


FIG. 55.

the shoulder, with the end turned up at right angles. A piece of platinum gauze should be wrapped round the tube at the point at which it is to be heated by the Bunsen flame.

*Preparation of the Substances to be Tested.* *Malt.*—50 gram. of the unground malt are digested at the ordinary temperature for 20 minutes with a mixture of 25 c.c. of the prepared hydrochloric acid free from arsenic and 75 c.c. of water. The acid solution is then decanted and 50 c.c. used for the test. With materials such as *Worts, Malt Substitutes, Commercial Malt Extract, Caramels, Brewing Sugars, Hops and Hop Substitutes, Yeast, Yeast Foods* and *Beer* experience has shown the necessity of destroying the organic matter. In the case of a solid an appropriate quantity, 10 gram. for example, is placed in a platinum dish and moistened with water, and 0.5 gram. arsenic-free calcium carbonate stirred in. The mass is heated until the charring and swelling are complete and then ignited at a low

temperature until free from carbon. A few c.c. of water are added, followed by 20 c.c. of the prepared hydrochloric acid and about 4 mg. of sodium bisulphite. When the calcium carbonate is dissolved the solution is filtered, made up to a known volume and an aliquot portion taken for the test. With liquids such as worts, and beer, 100 c.c. are evaporated to dryness with 0.5 gm. calcium carbonate, the subsequent procedure being the same.

*Hops* are best dealt with by measuring out 5 gm. and immersing for 15 minutes in 75 c.c. water; 25 c.c. of the arsenic-free hydrochloric acid are added and the digestion continued for a further 15 minutes. The hops are then squeezed and 40 c.c. of the filtered extract placed in a platinum basin and 2.5 gm. calcium carbonate added gradually. After evaporation to dryness the contents of the basin are charred, ignited and treated as in the case of beer.

*Anthracite and Coke.*—It is necessary to test the fuel used for drying malt and hops for arsenical contamination. Many methods have been suggested but the following, which has been worked out in the writer's laboratory, gives reliable results. The sample, taken in the usual manner by quartering, is ground in an iron mortar and passed through a 90 mesh sieve. 2 gm. are weighed into a porcelain boat and placed in a hard glass tube connected to an absorption apparatus containing glass beads moistened with arsenic-free hydrochloric acid. The tube in the region of the boat is heated to nearly a red heat and a fairly rapid stream of oxygen passed through until the contents of the boat cease to glow. From 8 to 10 litres of oxygen will be required for the combustion. When the apparatus has cooled the boat is removed and the ash transferred to a beaker heated nearly to boiling with 25 c.c. of hydrochloric acid, then diluted with water, filtered and stannous chloride solution (*vide supra*) added drop by drop until the solution becomes just colourless, and made up to 100 c.c. with water; 50 c.c. are transferred to the Marsh apparatus and the result expressed as "Fixed  $\text{As}_2\text{O}_3$ ." The absorption tube and combustion tube are washed several times with the contained acid diluted with about 50 c.c. of hot water in order to dissolve any volatile  $\text{As}_2\text{O}_3$  present, and finally rinsed with a little hot water. The dilute acid and washings are evaporated to about half volume, cooled, made up to 50 c.c. and placed in the Marsh apparatus, the result being expressed as "Volatile  $\text{As}_2\text{O}_3$ ." Up to 1914 maltsters and hop growers had little difficulty in obtaining fuel containing but

negligible traces of arsenic but since that time the purchaser has been largely at the mercy of his coal merchant and large quantities of anthracite and coke contaminated with arsenic have been used in malt kilns and roast houses. As a general rule, the "volatile arsenic" is the significant figure, the "fixed arsenic" remains in the ash of the kiln fire; it can, however, become volatile if any reducing material is added to the kiln, such as sulphur which is used more particularly with hops, for improving the colour of, and conferring brightness on, the product (*cf.* J. L. Baker and W. D. Dick, *J. Soc. Chem. Ind.*, 1904. 23, 174).

*Permissible Limits of Arsenic.*—The Royal Commission on Arsenical Poisoning (1903) were of opinion that no substance should be used as an ingredient of food which contained more than 1/100 grain per pound of arsenic, or in the case of solution of 1/100 grain per gallon. In many cases, more particularly glucose and malt, much smaller quantities than these are now commercially attainable, and should be insisted on.

*Method of Working.*—10 grm. of granulated zinc are placed in the flask, 25 c.c. of the purified hydrochloric acid run in and the evolution of hydrogen continued for 20 minutes. The burner is then lighted, the solution to be examined run in and the test allowed to proceed for 20 minutes. The volume of this solution should be 50 c.c. and its acidity adjusted so that it contains 1.25 grm. of hydrochloric acid.

*Preparation of the Standard.*—The standard mirrors with which the arsenic deposits from tested substances are to be compared in those cases where the organic matter is not destroyed must be prepared by the use of a specimen of each kind of substance containing known amounts of arsenious oxide. Every care should be taken that the period of time over which the solution is added, the size of the hydrogen flame, the mode and duration of heating of the glass tube, and the amount of acid used, should be the same in the preparation of the series of the standard mirrors as in the carrying out of the actual test. The mirrors as soon as deposited should be sealed at both ends in an atmosphere of hydrogen and kept in the dark. According to the experience of the writer the standard mirrors remain practically permanent for 3 months.

The standard arsenic solution is prepared by dissolving 0.1 grm. of pure arsenious oxide in a small quantity of pure strong hydro-

chloric acid. The liquid should not be heated. When the solution is complete it is diluted to 1000 c.c. with distilled water. 1 c.c. of of this solution contains 0.1 mg. of arsenious oxide.

## Malt Liquors

### BEER, ALE

*Beer* may be described as a fermented liquor brewed from malt or from a mixture of malt and malt substitutes and having a bitter flavour communicated by hops or by other wholesome bitter. In the Middle Ages, ale was a fermented infusion of malt and water flavoured with a small quantity of some bitter principle, such as oak bark. Beer, on the other hand, was made from malt, water and hops. The distinction between ale and beer lasted for a considerable time. Hops gradually came into general use, but the word ale was retained whether the liquor designated by it was hopped or not. The word "beer" now includes all liquors made from malt, whilst ale includes all but black or brown beers.

Under the present law of England, the malt used in brewing beer may be replaced by any sugar or amylaceous substance, and as the duty is levied on the quantity of soluble carbohydrates made into wort, as determined by the sp. gr. of the infusion, the exact nature of the fermentable matter employed is a matter of indifference to the Excise. Similarly, the employment of hops is not insisted on by the Excise, and any wholesome bitter (*e. g.*, quassia and gentian) can be employed. The substitution is not an infringement of the Sale of Food and Drugs Act, which could, however, be enforced in the case of a distinctly unwholesome bitter being used. It may, however, be pointed out that hop substitutes are only employed to a very slight extent in breweries; according to the Excise returns of last year 41,183,106 lb. of hops were used for brewing and only 8456 lb. of hop substitutes. As brewers are compelled by Excise regulations to declare the different materials and quantities they use it will be apparent how ill-founded are the irresponsible statements made from time to time in the press concerning the brewing of beer from "chemicals."

According to a recent German official statement (*J. Inst. Brew.*, 1921, 27, 125) the characteristic feature of beer is that it enables the consumption of a moderate amount of alcohol and other stimulating

products of fermentation in palatable and wholesome form, conditioned in part by the extractive matter (dextrin, sugar, etc.) and for the other part by the hop constituents, and the presence of carbonic acid generated by fermentation and retained probably in a characteristic form. If one of these constituents of beer is eliminated, the beverage loses its character and peculiar nature as beer.

The chemical composition of beer and other malt liquors is very complex; the two main constituents may be conveniently arranged in the following three classes:

(a) The volatile constituents, including alcohol, water, acetic acid, carbonic acid and some other acids.

(b) The fixed organic matters, forming the organic constituents of the "extract," including sugars, dextrins, glycerol, lactic and succinic acids, proteins and organic extractive matters from hops, etc.

(c) The mineral constituents or ash, consisting chiefly of potassium, calcium, and magnesium phosphates.

Beer differs from wine in its smaller content of alcohol, the presence of dextrin, and the greater proportion of extractive matters present; also in the absence of acid tartrates, which are characteristic of wine as malic acid is of cider and lactic acid of beer. The acidity of beer is frequently ascribed to acetic acid, but, except in sour ales, it is chiefly due to lactic acid, to other organic acids produced by fermentation, and acid phosphates.

The composition of malt liquors differs widely according to the nature and proportion of the materials used and the manner in which the fermentation has been conducted. Broadly speaking, two distinct methods of brewing are pursued, namely, the German (Lager) and the English. German beers are fermented at a low temperature, under which condition the yeast remains at the bottom of the liquid, and the process is said to be one of "bottom-fermentation." The yeast is a different variety from that of English breweries. Beer brewed on this system contains less alcohol and more dextrin, sugar, and nitrogenous matter than English beer, and hence is liable to undergo secondary fermentation unless kept at a very low temperature or else sterilised and preserved in bottles. The German beer also contains less hops than English beer. In the English system of brewing, the operation is one of "top-fermentation," and as a rule the product is richer in alcohol and contains less extractive matter than German beer.

Generally, bitter ales have a low racking gravity, high percentage of alcohol and much hop extract; mild ales higher racking gravities, less alcohol and less hop extract; porter about the same as mild ale, but less hops. Stouts usually have a high racking gravity and low alcohol content; they are hopped in proportion to their gravity. Export ales and stouts have low racking gravities and high content of alcohol and are heavily hopped. A lengthy list of the different beers of the world and their analyses may be found in Wahl and Henius' "Handy Book of Brewing and Malting." As this volume is out of print a few typical analyses of Lager and English beers are quoted.

ANALYSES OF LAGER BEERS (Grant, *J. Inst. Brew.*, 1909, 15, 385)

Data	Bottled English Lager		Bottled Imported Lager		Draught Imported Lager	
	(1)	(2)	(1)	(2)	(1)	(2)
Original gravity.....	1044.00	1050.00	1052.00	1042.00	1052.00	1053.00
Present gravity.....	1012.30	1016.90	1011.30	1015.60	1013.00	1020.70
Alcohol by weight, %.....	3.53	3.87	4.39	2.91	4.17	3.55
Extract (total solids).....	4.41	5.06	5.14	5.45	5.07	7.31
Ash in 100 c.c.....	0.270	0.278	0.178	0.153	0.21	0.20
Acidity (%) calculated as acetic acid (volatile).....	0.057	0.059	0.066	0.038	0.062	0.066
Acidity (%) calculated as lactic acid (fixed).....	0.085	0.088	0.099	0.057	0.093	0.099

ANALYSES OF ENGLISH BEERS (J. L. Baker, 1921)

Data	London Stout	London Porter	Dublin Stout	Burton India Pale Ale	London India Pale Ale	London Mild Ale
Original gravity.....	1057.2	1035.1	1054.4	1053.9	1054.8	1041.7
Present gravity.....	1017.1	1008.8	1015.4	1011.2	1013.9	1009.7
Alcohol by weight, %.....	4.2	2.7	4.0	4.4	4.3	3.3
Extract (total solids), %.....	6.1	3.4	5.6	4.8	5.2	3.9
Total acidity, (calculated as acetic).....	0.14	0.10	0.16	0.1	0.12	0.1

A full analysis of a beer is useful for technical control purposes, but given two beers brewed at the same gravity it is not possible, in the opinion of the writer, to adduce figures to show that one is of superior quality to the other. An analysis will give some information as to how a beer was brewed and it is also possible by the "Forcing Test," which will be described later, to form an idea as to the stability of a beer and how it will behave in the trade.

**Original Gravity of Beer Worts.**—As the duty on beer is calculated from the strength of the wort as indicated by its sp. gr. before fermentation, it becomes necessary to determine the original gravity in order to obtain the rebate allowed when the beer is exported. If the wort could always be examined in an unfermented state, it would merely be necessary to ascertain its sp. gr. and gauge its measure to obtain the data for calculating the allowance to be made. But by the process of fermentation the sp. gr. of the wort is diminished to an extent dependent on the amount of alcohol formed. The weight of alcohol produced being approximately 50% of the carbohydrate matter destroyed by the fermentation, it is evident that a determination of the alcohol in the fermented liquid would give the means of ascertaining the quantity of sugar destroyed, and hence of making the necessary correction for the reduction in the density of the wort (technically called its "attenuation") caused by the fermentation.

The practical details of the methods of estimating the original gravities of beer worts have been investigated by Graham, Hofmann, and Redwood, and more recently by Sir Edward Thorpe and Dr. H. Brown (*J. Inst. Brew.*, 1914, 20, 569) and the combined results show that the information can be obtained in the following manner:

**Distillation Method.**—The carbon dioxide and yeast if present are first removed from the sample of beer to be examined by filtering through paper. It is important that the funnel should be kept covered during this operation with a clock glass if filtration is at all prolonged in order to avoid loss of alcohol (J. L. Baker and H. F. E. Hulton, *J. Inst. Brew.*, 1915, 21, 389).

100 c.c. of the beer at a temperature of 60° F. are measured into a flask and introduced into the distilling flask (Fig. 56) of the original gravity apparatus with about 40 c.c. of wash water and distillation is continued until about 80 c.c. have passed over. The distillate is made up to 100 c.c. in the same flask at 60° F. and the sp. gr. determined. The residue in the distilling flask is cooled and transferred, together with washings, into the same 100 c.c. flask made up to volume with water at 60° F. and the sp. gr. ascertained. The sp. gr. of the distillate represents the fermented matter as a mixture of alcohol and water, and that of the residue the unfermented matter in the original wort. To find the amount of fermented matter the sp. gr. of the alcohol distillate

is subtracted from 1000 and the difference is the "spirit indication number" (see Table I). From this table the number of degrees of sp. gr. lost during fermentation which correspond to the "spirit indication" may be found. This number plus the sp. gr. of the unfermented matter represents the original gravity of the wort.

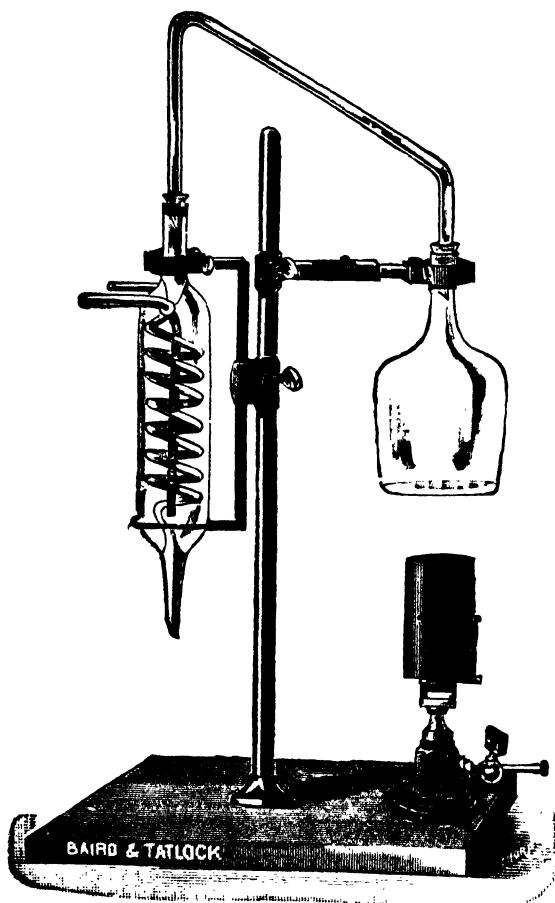


FIG. 56.

On the average, the table will allow original gravities to be determined accurately, but in some cases it will slightly overestimate and in others slightly underestimate original gravities. This is inevitable, as the true relation between spirit indication and degrees of gravity

lost varies with the composition of the wort and with the individual brewery. To meet cases where it may overestimate, the Finance Act allows a deduction of  $0.75^\circ$  from the gravity so ascertained for the purpose of the charge of duty. This, of course, applies to work connected with the departments of Customs and Excise. For purposes of brewery control, it may be found that a smaller correction, or one of contrary sign, or none, is needed.

TABLE I  
SPIRIT INDICATION TABLE SHOWING DEGREES OF GRAVITY LOST  
IN MALT WORT DURING FERMENTATION<sup>1</sup>

Degrees of spirit indication	0 0	0 1	0 2	0 3	0 4	0 5	0 6	0 7	0 8	0 9
0	0 00	0 42	0 85	1 27	1 70	2 12	2 55	2 97	3 40	3 82
1	4 25	4 67	5 10	5 52	5 95	6 37	6 80	7 22	7 65	8 07
2	8 50	8 94	9 38	9 82	10 26	10 70	11 14	11 58	12 02	12 46
3	12 90	13 34	13 78	14 22	14 66	15 10	15 54	15 98	16 42	16 86
4	17 30	17 75	18 21	18 66	19 12	19 57	20 03	20 48	20 94	21 39
5	21 85	22 30	22 76	23 21	23 67	24 12	24 58	25 03	25 49	25 94
6	26 40	26 86	27 32	27 78	28 24	28 70	29 16	29 62	30 08	30 54
7	31 00	31 46	31 93	32 39	32 86	33 32	33 79	34 25	34 72	35 18
8	35 65	36 11	36 58	37 04	37 51	37 97	38 44	38 90	39 37	39 83
9	40 30	40 77	41 24	41 71	42 18	42 65	43 12	43 59	44 06	44 53
10	45 00	45 48	45 97	46 45	46 94	47 42	47 91	48 39	48 88	49 36
11	49 85	50 35	50 85	51 35	51 85	52 35	52 85	53 35	53 85	54 35
12	54 85	55 36	55 87	56 38	56 89	57 40	57 91	58 42	58 93	59 44
13	59 95	60 46	60 97	61 48	61 99	62 51	63 01	63 52	64 03	64 54
14	65 10	65 62	66 14	66 66	67 18	67 70	68 22	68 74	69 26	69 78
15	70 30	70 83	71 36	71 89	72 42	72 95	73 48	74 01	74 54	75 07
16	75 60									

<sup>1</sup> The Finance Act of 1914 (Session 2) substitutes this for the former table. G. C. Jones and J. L. Baker have computed the table to hundredths of a degree in a form suitable for laboratory use (Published by the *Brewers' Journal*, London, 1914).

The experimental data on which the table was constructed included the formation of  $0.1\%$  acidity calculated as acetic acid, and no correction is necessary in the case of beers containing about this proportion. Any excess of acidity over  $0.1\%$  is supposed to be formed at the expense of the alcohol in the beer, and unless this acidity is allowed for the original gravity will be low. The amount of acid present in the beer is estimated by titration with  $N/10$  ammonium hydroxide using litmus paper as an indicator. From the result  $0.1\%$  is subtracted and the difference referred to Table II which indicates the correction due to the excess of acid formed. This number is then added to the spirit indication figure.

When a beer has become very acid with acetic acid it is necessary to make allowance in respect of it; firstly, because some of the alcohol formed has been lost by being converted into acetic acid and, secondly,

TABLE II  
TABLE FOR ASCERTAINING THE CORRECTION FOR ACID

Excess per cent. of acetic acid in beer	Corresponding degrees of spirit indication									
	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.0	....	0.02	0.04	0.06	0.07	0.08	0.09	0.11	0.12	0.13
0.1	0.14	0.15	0.17	0.18	0.19	0.21	0.22	0.23	0.24	0.26
0.2	0.27	0.28	0.29	0.31	0.32	0.33	0.34	0.35	0.37	0.38
0.3	0.39	0.40	0.42	0.43	0.44	0.46	0.47	0.48	0.49	0.51
0.4	0.52	0.53	0.55	0.56	0.57	0.59	0.60	0.61	0.62	0.76
0.5	0.65	0.66	0.67	0.69	0.70	0.71	0.72	0.73	0.75	0.64
0.6	0.77	0.78	0.80	0.81	0.82	0.84	0.85	0.86	0.87	0.89
0.7	0.90	0.91	0.93	0.94	0.95	0.97	0.98	0.99	1.00	1.02
0.8	1.03	1.04	1.05	1.07	1.08	1.09	1.10	1.11	1.13	1.14
0.9	1.15	1.16	1.18	1.19	1.21	1.22	1.23	1.25	1.26	1.28
1.0	1.29	1.31	1.33	1.35	1.36	1.37	1.38	1.40	1.41	1.42

because the acetic acid will distil over with the alcohol and raise the sp. gr. of the distillate and consequently reduce the apparent spirit indication and also the original gravity. In such cases, Moritz and Morris (*Text-book of the Science of Brewing*, E and F. N. Spon, p. 503) advise that a second distillation be performed in presence of sufficient alkali to neutralise the acid and so prevent the distillation of the acetic acid. The sp. gr. of the residue in such a case is determined on another portion of the beer boiled without the addition of alkali. The method of calculation will be seen from the following example:

Total acidity of beer (as acetic acid).....	0.14%
Sp. gr. of water at 60° F.....	1000.0
Sp. gr. of distillate at 60° F.....	991.94
Difference = " <i>spirit indication</i> ".....	8.06
Allowance for alcohol corresponding to (0.14 - 0.10 =) 0.04% excess of acid.....	0.07
<i>Corrected spirit indication</i> .....	8.13
Equal, by table, to " <i>gravity lost</i> ".....	1036.25
To which add sp. gr. of extract.....	1031.16
<i>Original gravity of wort</i> .....	1067.41
Sp. gr. of beer (apparent gravity).....	1023.20
Check (23.20 + 8.06) =	1031.26.

A useful "check" on the accuracy of an original gravity determination consists in adding the sp. gr. of the unboiled beer (apparent gravity) to the spirit indication. The figure so obtained should equal (or very slightly exceed) the extract or residue gravity. If there be any discrepancy, it will usually be found that the error lies in the value found for the apparent gravity.

The table already given (page 205) is the only one legalised by the Finance Act of 1914 for the determination of original gravities, and is used by the Excise, whether the wort be derived wholly or partly from starch—or cane-sugar, or simply from malt.

*The Optical Method of Determining Alcohol and Extract in Beer.*—At the request of the Norwegian Government, H. Tornøe undertook the task of devising a rapid and simple method of beer analysis for revenue purposes. He elaborated a process whereby the amount of alcohol and extract in a beer can be ascertained in about 10 minutes. The measurements involved are the sp. gr. of the beer at 63.5° F. and the index of refraction of the beer for sodium light at the same temperature. Ling and Pope (*J. Fed. Inst. Brew.*, 1901, 7, 170) describe the following method of carrying out the determination.

The refractometer, lens, and sodium lamp are arranged in a line, the distance between the axis of the instrument and the lens being about 12 in., whilst that between the sodium flame and the axis of the instrument is about 27 or 28 in. The cross-wires of the telescope are sharply focussed by sliding the eye-piece slightly in or out, and the refractometer is then adjusted. This is done by gently pressing the telescope carrier against the screw by means of the thumb and finger, when the zero of the outer or vernier scale should correspond exactly with the 180 division of the inner scale; if this be not the case, the adjustment is readily made by slightly turning the screw in one direction or the other as is required. The table which carries the prism is next placed on the axis of the refractometer and turned round until the lever points approximately to the division 40 of the inner divided circle.

The glass prism, previously cleaned and dried, is now set on the round table in such a way that it is firmly fixed by the springs provided for that purpose, the ground glass edge of the prism being towards the lever. It is important that the angles formed by the meeting of the divided partition of the prism with the two parallel end walls should be free from dirt.

The beer to be examined, after the carbonic acid has been removed by filtration through paper, is poured into the right-hand division of the prism, and water of the same temperature, which should not differ much from  $63.5^{\circ}$  F., into the left-hand division, the surfaces of the two liquids being about  $\frac{1}{4}$  in. from the top of the prism.

The telescope, while still pressed gently with the left hand against the screw is moved slowly round towards the right hand of the observer, while the lever is also slowly moved in such a manner as to keep the dividing partition of the prism directed towards the sodium flame. The eye of the observer being meanwhile applied to the telescope, it will be found that, as the telescope is moved round, the left-hand portion of the field of view becomes darkened; the line of demarcation between the bright and dark halves of the field is then adjusted by a slight movement of the lever in one direction or the other until it exactly passes through the point where the cross-wires cut. When this has been done, the lever must not be moved again during the remainder of the measurement.

The prism is next turned through an angle of  $180^{\circ}$ , so that the positions of the beer and water become interchanged, and the partition again points towards the sodium flame; this is done by means of a lever which is moved round to the left of the observer, the telescope being at the same time held in about its original position. The telescope is then moved slowly round to the left of the dividing partition of the prism, until once again the field is divided into light and dark halves, the telescope being so adjusted that the dividing line passes through the point of intersection of the cross-wires; in this case it will be noticed that the left-hand side of the field is light and the other dark.

In order to make sure that the correct position is obtained, the whole refractometer may be moved slightly to and fro by means of the lever, when the dividing line should not move relatively to the cross-wires. When the telescope has been exactly adjusted, the vernier scale and the inner graduated circle will be found to be in contact, and the angle  $2$ , corresponding to the zero point of the vernier, is read off directly either with the naked eye or by means of a lens. Finally, the prism is once again rotated back into its original position, and the telescope brought into contact with the screw, when the dividing line should pass through the middle of the field; if this

be not the case, the prism must have been displaced during the operation, and the reading must be repeated.

J. Race (*J. Soc. Chem. Ind.*, 1908, **27**, 544) has applied the immersion refractometer (see p. 29) to the estimation of alcohol and extract in beer. The process is based on the fact that if the specific gravity and refractive index are known, the alcohol and extract percentages may be calculated, for if  $C_1$  be the amount by which the refractive index is raised by each 1% of alcohol,  $C_2$  the amount by which it is raised by each per cent. of extract,  $C_3$  the amount by which the sp. gr. is lowered by each per cent. of alcohol, and  $C_4$  the amount by which it is raised by each per cent. of extract, and  $X$  and  $Y$  are the percentages of alcohol and extract required—

$$\left. \begin{array}{l} \text{then } r, \text{ the ref. index of the sample} \\ \text{index of water} \end{array} \right\} = C_1X + C_2Y,$$

$$\left. \begin{array}{l} \text{and } s, \text{ the sp. gr. of the sample} \\ \text{of water (1.0)} \end{array} \right\} = -C_3X + C_4Y,$$

from these it follows that—

$$X = \frac{C_4r - C_2s}{C_2C_3 + C_4C_1} \text{ and } Y = \frac{C_3r + C_1s}{C_2C_3 + C_4C_1}$$

Barth (*Zeitsch. ges. Brauw.*, 1905, **28**, 303-306; *J. Soc. Chem. Ind.*, 1905, **29**, 630) made a number of determinations of the values of  $C_1$ ,  $C_2$ ,  $C_3$ ,  $C_4$ , and the figures so obtained substituted in the above expressions give the following formulæ:

$$X = [0.007598r - 0.002923s] 100,000$$

and

$$Y = [0.003366r + 0.001303s] 100,000$$

For samples containing under 4.5% alcohol the Barth formula, according to Race, gives satisfactory results but over that figure the tendency is to give rather low results for both  $X$  and  $Y$ . For such samples the formula

$$X = [0.00778r - 0.0020s] 100,000$$

$$Y = [0.0035r + 0.001305s] 100,000$$

is to be preferred.

The modified formulæ give on the average 0.5° to 0.7° higher original gravity. The original gravity is calculated in the usual way from the alcohol and extract figures by the use of spirit indication

tables. There is no doubt that the refractometer method gives results which are quite as accurate as those obtained by distillation, and as much less time is required the method is very useful in those laboratories where many Original Gravity determinations have to be made. In any dispute the official distillation method must be used.

*Estimation of Alcohol.*—The amount of alcohol in a fermented wort or beer is obtained by referring the sp. gr. of the alcoholic distillate such as is obtained in the original gravity determination (see page 143) to the tables (see pages 144 to 151) which show the weight of alcohol corresponding to a given sp. gr. of aqueous alcohol; from the weight found that of alcohol in 100 c.c. of the original beer is calculated.

*Estimation of Extract.*—The proportion of extract or matter remaining in a beer may be deduced from the sp. gr. of the de-alcoholised liquid obtained by evaporating the sample to one-third and diluting again to its original volume. The sp. gr. of the extract is then observed, and the excess above 1000 divided by 4, the quotient being the number of grams of dry extract contained in 100 c.c. of the beer. Or the residue left in the distillation flask in the original sp. gr. determination may be used for this purpose. The "apparent maltose" and "apparent dextrin" may be determined in the extract in the manner described on page 183.

*The Amount of Unfermentable Matter in a Beer.*—This determination is of value in forming an opinion as to the probable course of the so-called "secondary" fermentation, and the resulting condition of the beer. When systematically made it also affords information of the suitability of the pitching yeast used in a brewery.

100 or 200 c.c. of the beer are evaporated until the alcohol is removed, made up to the original volume and fermented with 1 or 2 grm. of pressed yeast for 48 hours. The solution is then boiled to expel alcohol, made up to the original volume with water and a small quantity of alumina cream and filtered. The grams of maltose per 100 c.c. are then determined from the reducing power and the difference in the amount of maltose before and after fermentation represents the amount of fermentable matter remaining in the beer. This difference, although conveniently expressed as maltose, includes fermentable low-type malto-dextrins, etc.

*Total Nitrogen.*—This may be estimated by the Kjeldahl process. 25 c.c. of the beer are evaporated with a few drops of sulphuric acid to a small bulk in the flask in which the decomposition with sulphuric acid is carried out, and the whole taken nearly to dryness and then heated with 20 c.c. of strong sulphuric acid and potassium sulphate in the usual way. The nitrogen multiplied by the factor 6.25 gives the total protein percentage.

*Colour.*—The colour of beers is determined in the Lovibond tintometer with the precautions as described under "Malt Analysis" (page 174). If the beer is not brilliant, a few drops of finings may be added or not more than one or two drops of alumina cream per 100 c.c. of beer lest loss of colour should result. No clarifying agent whatever must be employed in the case of black beers, and as these are too dark to be read even in a  $\frac{1}{2}$ -in. cell, they must be suitably diluted and the necessary correction applied.

*Estimation of Carbon Dioxide in Beer.*—Since carbon dioxide contributes to the "palate" and plays a considerable part as a natural preservative in beer its estimation is of some importance. The following method has been worked out in the writer's laboratory. The apparatus (Fig. 57) consists of a boiling vessel (100 c.c. pipette with ends cut off) attached above to a nitrometer (50 c.c.) with a funnel top and below by about 40 in. of pressure tubing to a mercury reservoir of about 200 c.c. capacity. Pinchcocks are used to close the connections between the boiling vessel and the measuring and the mercury vessels. The boiling and measuring vessels are filled with mercury, part of which is replaced through the funnel with the sample to be examined (usually about 25 c.c.). The mercury vessel is lowered so as to bring the beer down into the lower part of the boiling vessel, and this is well shaken. The lower pinchcock is closed and the mercury is raised. Then the lower pinchcock is opened cautiously and the beer raised till it just reaches the upper

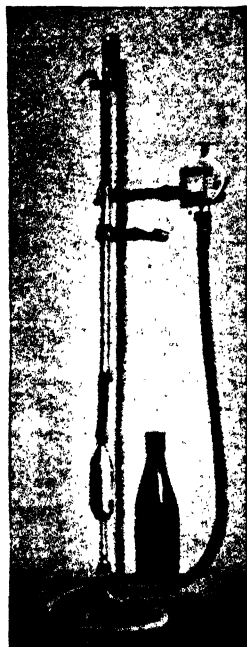


FIG. 57.

pinchcock. This is closed and the beer lowered and again shaken and gently boiled for 5 minutes. The gas liberated is passed into the measuring vessel, by raising the mercury and letting it through the upper pinchcock, and the process repeated until the amount of gas separated becomes negligible. The apparatus is allowed to cool, the pressure adjusted and the volume of gas read off in the nitrometer.

English ales of original gravity of  $1045^{\circ}$  to  $1050^{\circ}$  contain 600 to 800 c.c. of  $\text{CO}_2$  per litre; stouts in good condition may exceed 1000 c.c. per litre, and beers in excessive condition may contain 2500 to 3000 c.c. per litre. A beer containing less than 500 c.c. per litre is "flat" to the palate.

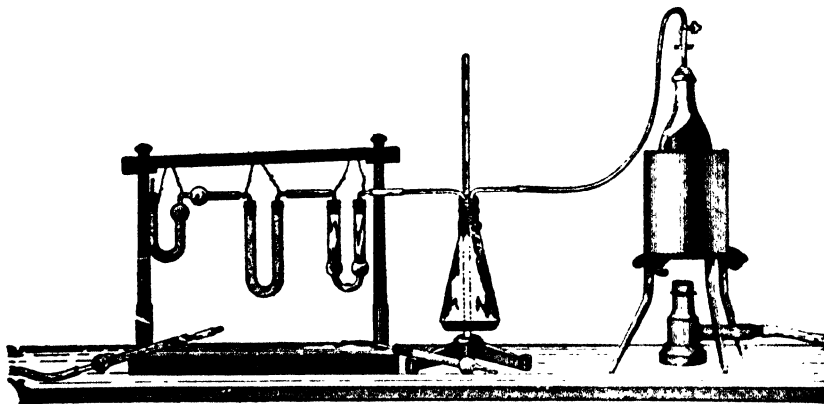


FIG. 58.—Crampton and Trescot; Bulletin 107, Bur. of Chem. U. S. Dept. Agric.

*Bottled Beers.*—The cork is pierced with a champagne tap. (Crampton found it advantageous to re-grind the cocks and ream off the thread.) The bottle thus tapped is connected with the absorption apparatus shown in Fig. 58, devised by Crampton and Trescot. The can containing the bottle holds a convenient amount of cold water. The tap is opened so as to allow the gas to escape slowly, and when the flow ceases, the water is heated slowly to about  $80^{\circ}\text{C}$ ., shaking the bottle from time to time during about 30 minutes while this temperature is maintained. The bottle is then disconnected and air under the usual precautions drawn through the apparatus. The increase in weight of the absorption tube gives the amount of carbon dioxide. The contents of the bottle are either

weighed or measured to give the necessary data for calculation. Bottles carrying patent stoppers can sometimes be adapted to this method by substituting quickly a rubber stopper fitted with a suitable stopcock tube. When this cannot be done, the method given in the next paragraph must be employed.

A round-bottom flask, about 700 c.c. capacity, is provided with a rubber stopper carrying two stopcock tubes, each bent at right angles, one tube passing to the bottom of the flask, the other terminating just below the stopper. A partial vacuum is produced in the flask which is then weighed. The end of one of the stopcock tubes is then dipped below the surface of the sample, and about 300 c.c. allowed to enter the flask, which is then weighed, and the procedure for bottled samples followed. It is recommended as better manipulation to attach to one of the stopcock tubes, by means of a rubber tube, a champagne tap that has been screwed into the cask. Somewhat better results may be obtained by placing a reflux condenser between the flask and absorption apparatus and heating the flask until the contents boil.

*The Mineral Constituents of Beers.*—The total ash of a beer may be estimated by evaporating 50 c.c. of the sample to dryness in a large platinum or silica basin, and cautiously igniting at a low red heat in a muffle. An estimation of certain of the ash constituents is sometimes useful in determining if a beer is correctly described as the product of a certain brewery. For example, a brand of beer may be brewed to contain certain proportions of gypsum or chlorides. An estimation of these constituents would afford material evidence in detecting a case of fraudulent substitution.

In instances where the addition of large quantities of sodium chloride is suspected a quantity of the beer (50 c.c.) should be evaporated to dryness in the presence of sodium carbonate and ashed in a muffle at as low a heat as possible. The total chlorine in the ash is estimated gravimetrically. Race (*J. Soc. Chem. Ind.*, 1908, **27**, 548) recommends evaporating 50 c.c. of the beer with 0.5 gm. of barium carbonate and subsequently igniting to a black ash. The ash is extracted with hot water, filtered, and titrated with silver nitrate in the usual manner.

Another volume of 50 c.c. of the beer is evaporated to dryness, moistened with sulphuric acid and ashed. The potassium is estimated as chloriplatinate and the sodium by difference. From these

data and the chlorine the amount of sodium chloride present in the beer may be calculated. It is convenient to express the chlorine found in terms of sodium chloride. It is customary in brewing ale to add chlorides either to the liquor or to the copper when the water is deficient in these constituents in order to impart a certain palate fullness. The total chlorides (as sodium chloride) in a beer of an original gravity of 1050° should not greatly exceed 50 grains per gallon. Of this amount 20 to 25 grains may be derived from the materials (malt, hops and water).

*Sulphate.*—50 c.c. of the beer are evaporated to dryness in the presence of a small quantity of sodium hydroxide, the mass ashed and the sulphates estimated in the usual manner. If the beer is burnt by itself loss of sulphuric anhydride occurs owing to the interaction between acid phosphates and the sulphates.

The question of whether a beer is made from an all-malt grist or part malt and part substitute is often asked. It may be asserted that with a few exceptions nearly all brewers use substitutes. In the United Kingdom the malt comprised, in 1919, 92% of the solid materials used in brewing, the remaining 8% including unmalted corn, malt substitutes, and sugars. The nature and proportion of nitrogenous matter has been suggested as a means of detecting substitutes, but when the varying composition of malt is borne in mind it will be realised that no safe conclusion can be drawn from such data. The fact that that amount of phosphoric acid is higher in an all-malt beer than in one brewed with substitutes has been proposed as a means of detecting substitutes. The amount of phosphates, however, differs in malt, also the quantity taken up in the development of the yeast is not constant. Hence the evidence afforded by this estimation is only diagnostic and not conclusive.

*Detection of Bitter Substances in Beer.*—Elaborate processes have been devised by Dragendorff, Wittstein and others for detecting the presence of substances which might possibly be used for imparting a bitter taste to beer, but it will be sufficient to describe here the method of searching for the more commonly used "hop surrogates," and certain objectionable substances the occasional employment of which is suspected.

A. C. Chapman (*Analyst*, 1900, 25, 35) has devised a method for distinguishing between hops and quassia, which is based upon the production of valeric acid when an ethereal extract of hops is oxi-

dised with an alkaline solution of potassium permanganate. 500 c.c. of the beer are evaporated on the water-bath with the addition towards the end of the operation of some ignited sand, the mass being constantly stirred to prevent it from adhering to the surface of the dish. The residue is dried in an air-oven, finely powdered and extracted in a bottle with ether. The ether is removed from the extract and the residual matter oxidised by the careful addition of an alkaline solution of potassium permanganate containing 40 grm. of permanganate and 10 grm. of potassium hydroxide in 1000 c.c. This solution should be added in small quantities at a time, the flask being vigorously shaken and if necessary warmed. When the permanganate ceases to be readily reduced, a few drops of a hot solution of oxalic acid are added to complete the reduction, and the colourless liquid filtered from the manganese oxides into a glass dish, in which it is evaporated to dryness. The dry residue is then acidified with dilute sulphuric acid, when the odour of valeric acid in the case of the hop-bittered liquid becomes at once apparent, being rather accentuated by the carbon dioxide liberated at the same time from the potassium carbonate formed during the oxidation. The smell observed is not that of pure valeric acid, but of valeric acid plus some other odourous compound, which serves to render it more characteristic. In the case of the quassia the liberated acid is chiefly acetic. Old hops respond as readily to this test as new hops. Camomile extract behaves in a similar manner to hops, but chiretta yields no valeric acid.

This method is applicable to the examination of hop-bitter preparations (of a medicinal character), hop extracts, and similar products. It furnishes additional evidence in the case of fermented beverages which have been examined according to the systematic schemes in vogue, and which have yielded results of an uncertain nature.

*Preservatives in Beer.*—The most commonly occurring preservative is sulphurous acid, usually as a sulphite or, rarely, in the free state. Salicylic acid is also used, often in association with a sulphite. Fluorides are occasionally found in continental beers.

*Salicylic Acid.*—The following process devised by F. T. Harry and W. R. Mummery (*Analyst*, 1905, 30, 124-127) gives satisfactory results:

100 c.c. of the beer are placed in a graduated 200 c.c. flask made alkaline with 5 c.c.  $N/1$  sodium hydroxide and the alcohol driven

off at a temperature just below the b. p. After cooling 5 c.c. of normal hydrochloric acid are added and 20 c.c. of basic lead acetate solution; the mixture is then made alkaline with about 20 c.c. of N/1 sodium hydroxide and made up to 200 c.c. At this stage the solution may be raised to boiling and allowed to cool before filtering, but this may be omitted if thought advisable. 100 c.c. of the filtrate are acidified with hydrochloric acid, a precipitate of lead chloride being thrown down and filtered off. The filtrate is extracted with ether three times, the ether distilled off and the salicylic acid dissolved in a small quantity of dilute alcohol and made up to 100 c.c. The salicylic acid is estimated colourimetrically in ordinary 50 c.c. Nessler tubes with very weak ferric chloride solution which should be made up freshly when required. The standard salicylic solution is 0.01% strength. The tendency which beers have to emulsify when shaken with ether is obviated by this process.

**Sulphites.**—J. L. Baker and F. E. Day (*J. Inst. Brew.*, 1911, **17**, 465; also *Analyst* 1912, **37**, 439) investigated the question of the estimation of sulphites in beers, wines, etc., and suggested a procedure which has given reliable results. The apparatus consists of a litre flask fitted with a dropping funnel and connected to a condenser. To the receiver end of the condenser are connected two absorption flasks of about 120–150 c.c. capacity. The delivery tubes to these flasks are provided with large bulbs to check regurgitation. About 100–150 c.c. of water and 20 grm. of clean white marble chips are placed in the large flask, and a known volume (5 c.c.) of N/10 iodine in the absorption flasks, the bulk in the first, the last few drops, diluted with 0.5% potassium iodide solution, in the second. About 10 c.c. hydrochloric acid are added by means of the dropping funnel, and the contents of the flask brought to the boil to allow all traces of air to be driven from the apparatus. After a few minutes the beer is added cautiously from the dropping funnel, the rate of addition and the flame under the flask being regulated so that neither frothing of the contents of the flask nor the drawing of air through the absorption apparatus occurs. Boiling is continued for about half an hour, during which time freshly boiled dilute hydrochloric acid (1:3) is allowed to drop into the flask, so as to maintain a gentle current of CO<sub>2</sub> through the absorption flasks. Care has to be taken not to introduce air during these additions. At the expiration of

the half-hour about 100 c.c. of distillate has been collected; the apparatus is then disconnected and the excess of iodine titrated with N/10 thiosulphate.

Using this apparatus, it is possible to distil 400–500 c.c. of beer in successive quantities before excessive frothing renders disconnection and emptying the distillation flask necessary. Any solution added to the beer before or during distillation should be freshly boiled, as sufficient oxygen may be dissolved to oxidise an appreciable amount of sulphurous acid.

*Fluorides.*—Bolton and Revis (*Fatty Foods*: J. and A. Churchill, p. 109) have described a method for the detection and approximate estimation of fluorides in margarine, which is applicable, with certain modifications, to malt liquors. 100 c.c. of the beer is rendered slightly alkaline with sodium carbonate, evaporated to dryness and ignited. To the residue are added 30 c.c. of a 20% solution of acetic acid, and the whole allowed to boil on a sand-bath for 15 minutes, covered with an inverted funnel. The contents of the basin are then washed on to a small filter with hot acetic acid solution, and the filter dried and ignited in a platinum crucible and weighed. The residue is mixed with a few drops of pure concentrated  $\text{H}_2\text{SO}_4$ , and the crucible covered with a waxed watch-glass on which some design has been scratched with a pin. The watch-glass is filled with water and the crucible heated on a hot plate or the top of a water-bath for 30 minutes. The glass is then removed and examined for etching. If fluorides are indicated the crucible is then ignited *very cautiously in an inclined position* and re-weighed. The fluorides having been converted into sulphates by the operation, the amount present may be approximately calculated, since 78 parts of  $\text{CaF}_2$  give 136 parts of  $\text{CaSO}_4$ . It is assumed that all the residue is calcium sulphate derived from calcium fluoride. In exact work the calcium sulphate should be estimated in 100 c.c. of the beer and the amount found subtracted from the weight of  $\text{CaSO}_4$  found in the test.

*Saccharin* (Benzoic Sulphimide).—The addition of saccharin is forbidden in most countries. Allen (*Analyst*, 1888, **13**, 105) devised the following method for its detection:

The beer is concentrated to one-third its volume, and if not acid, is rendered so by the addition of a little pure phosphoric acid. The liquid is then shaken with ether, the ether decanted and evaporated,

and the residue burned off after being mixed with sodium carbonate and a little sodium nitrate. The sulphur in the saccharin is thus converted into sulphate, and can be estimated in the usual way. The weight of barium sulphate multiplied by 0.785 gives the weight of saccharin. Of course, all the reagents must be free from sulphates.

According to Bonis (*Ann. Falsif.*, 1917, 10, 210-218; *Analyst*, 1917, 42, 303) the saccharin is best separated by extracting the acidified substance with ether; in the case of alcoholic liquids the alcohol must be removed previous to the extraction, and in many instances a preliminary treatment with lead acetate and subsequent removal of the excess of lead will be found advantageous in eliminating substances which might contaminate the extracted saccharin. The residue obtained on evaporating the ether will consist of saccharin still contaminated with traces of tannin, colouring matters, salicylic acid, etc.; it is purified by dissolving in a few c.c. of dilute sulphuric acid, heating the solution on a water-bath and adding concentrated permanganate solution, drop by drop, until a slight excess is present. The mixture is then cooled, extracted with petroleum spirit, and the extract evaporated. The saccharin thus separated may be identified as such by fusing a portion of it with sodium hydroxide for 1 minute at 270° C., dissolving the mass in water, acidifying the solution with sulphuric acid, and extracting it with petroleum spirit. The latter extracts the salicylic acid formed from the saccharin, and the salicylic acid may be identified by the ferric chloride test. Several confirmatory tests may be applied. Saccharin yields a purple colouration when heated with phenol and concentrated sulphuric acid and the mixture then rendered alkaline with sodium hydroxide. A blue colouration is obtained when saccharin is heated with sulphuric acid and the solution then treated with sodium phenolate solution and sodium hypochlorite solution. The weight of the purified saccharin residue gives approximately the quantity of the substance present. If desired, the amount of saccharin may be estimated in this residue by fusing it with sodium hydroxide, extracting the salicylic acid formed, and estimating this colourimetrically with ferric chloride. A control test must, however, be carried out at the same time and under exactly similar conditions, using a known quantity of pure saccharin.

*The Stability of Finished Beers.*—Useful information as to the keeping qualities of beers and their suitability for certain purposes,

such as bottling, exporting to hot countries, etc., may be obtained from the "forcing test." (For a full description of this test, which was originally devised by H. T. Brown, see Matthews and Lott, "*The Microscope in the Brewery*," 2nd ed., page 128.) Formerly it was customary to place the beer to be examined in a small flask fitted with a side tube which dipped into mercury. The more general practice now is to fill a carefully cleaned screw-stoppered bottle made of white glass with the beer to be tested and keep in a suitable incubator or cupboard at a temperature of 80° F. for certain specified periods which are regulated by the "trade expectations" of the beers under examination. The appearance, apparent gravity, acidity, flavour, odour and condition are recorded before and after the test and the sediment which is formed carefully examined for wild yeasts and bacteria.

Beers for export purposes and stock ales and stouts should be perfectly sound and the sediment free from bacteria after 4 weeks' duration of the test. India pale ales should stand 3 weeks; light bottling ales and stouts a fortnight; and running beers, such as mild ale and porter, a week. The loss in gravity during the forcing test is an indication as to the rapidity with which a beer will get into condition in bottle. It is not possible to discuss the test fully in the present article, but if intelligently used it affords information of considerable diagnostic value to the brewer.



# WINES AND POTABLE SPIRITS

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Revised by LEWIS EYNON, B. Sc., F. I. C.

## WINES

Many distinguished chemists have devoted attention to the analysis of wines and new methods or modifications of old ones are proposed annually. Since, however, few of the estimations are absolute, new methods, even if good, are very cautiously received by continental chemists, who hold that it is more important to obtain numbers strictly comparable with those previously accumulated than to increase slightly the accuracy of a single determination. In these circumstances, the reader has a right to expect in a work of this kind a description of official methods of analysis, and with this in mind the writer has tried to steer a middle course between rival official methods, and, where the estimation is one which may be seriously influenced by departure from standard conditions, to point out differences in the official methods of different countries. Frequent reference is made in this section to "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists" revised to Nov. 1, 1919, and published by the Association in the United States. The methods described in this publication, which is referred to by the abbreviation A. O. A. C., differ very little from the German methods, which may be found described in great detail in K. Windisch's *Chemische Untersuchung des Weines* (Berlin, 1896). A German imperial decree of 1896 was very fully abstracted in *J. Soc. Chem. Ind.*, 1898, **17**, 277, and will be found to contain an amount of detail concerning analytical methods which is scarcely justifiable in this work.

The following determinations are usually made:

Specific gravity.

Alcohol.

Glycerol.

Extract.

Ash.

Acidity (volatile and fixed).

Sugar.

Potassium sulphate.

Sulphurous acid.

In addition to these it is sometimes necessary to estimate tannin and to look for saccharin, salicylic acid and other preservatives. It is convenient to return all results as grm. per 100 c.c. of wine. The determination of the sp. gr. and estimation of alcohol do not need description here.

**Glycerol.**—The German official methods are as follows:

(a) In wines containing less than 2 grm. of sugar per 100 c.c., 100 c.c. are evaporated to 10 c.c. in porcelain on the water-bath, and the residue mixed with 1 grm. of quartz sand and, for each gram of extract present, 1.5 to 2 c.c. of milk of lime (40% calcium hydroxide). Evaporation is continued almost to dryness and then 5 c.c. of 96% alcohol added. The matter which adheres to the sides of the dish is loosened with a spatula and reduced by a small pestle to a thin paste, further small quantities of 96% alcohol being added as required. The mixture is heated on the water-bath, with constant stirring, until it begins to boil, when the liquid portion is decanted into a 100 c.c. flask. The residue in the dish is repeatedly extracted with 10 c.c. portions of 96% alcohol, which are decanted into the 100 c.c. flask, until this contains about 95 c.c. The contents of the flask are cooled to 15° and 96% alcohol added until the volume reaches 100 c.c. The liquid is filtered and 90 c.c. evaporated in porcelain on a water-bath, avoiding vigorous boiling of the alcohol. The residue is taken up with a small quantity of absolute alcohol which is poured into a stoppered cylinder, and the dish washed out with more alcohol until the cylinder contains 15 c.c. Three separate portions of absolute ether, each of 7.5 c.c. are now added to the contents of the cylinder, which is vigorously shaken after each addition. When the liquid appears quite clear, it is poured into a tared glass dish, the cylinder rinsed with 5 c.c. of alcohol-ether mixture (2:3) and the rinsings added to the dish, which is then placed on a hot water-bath, which, however, must not be so hot as to cause actual boiling of the liquid. The syrupy residue is dried in the steam-oven for an hour, cooled in a desiccator and weighed.

(b) In wines containing more than 2 grm. of sugar per 100 c.c., 50 c.c. are warmed in a capacious flask on the water-bath, and mixed with 1 grm. of quartz sand and small quantities of milk of lime added till the colour, at first dark, again becomes pale and the liquid assumes a characteristic alkaline odour. After cooling, 100 c.c. of 96% alcohol are added, the precipitate allowed to subside, and the liquid filtered, the precipitate and filter being washed with 96% alcohol. The filtrate is then treated as in process (a).

The official A. O. A. C. methods by direct weighing, which differ only in detail from the German methods, are as follows:

*Dry Wines.*—Evaporate 100 c.c. of the wine in a porcelain dish on the water-bath to a volume of about 10 c.c., and treat the residue with about 5 grm. of fine sand and 4 to 5 c.c. of milk of lime (containing about 15% of calcium oxide) for each gram of extract present, and evaporate almost to dryness. Treat the moist residue with 50 c.c. of 90% alcohol by volume, remove the substance adhering to the sides of the dish with a spatula and rub the whole mass to a paste. Heat the mixture on a water-bath, with constant stirring, to incipient boiling and decant the liquid through a filter into a small flask. Wash the residue repeatedly by decantation with 10 c.c. portions of hot 90% alcohol until the filtrate amounts to about 150 c.c. Evaporate the filtrate to a syrupy consistency in a porcelain dish on a hot, but not boiling, water-bath; transfer the residue to a small, glass-stoppered, graduated cylinder with 20 c.c. of absolute alcohol and add 3 portions of 10 c.c. each of anhydrous ether, shaking thoroughly after each addition. Leave until clear, then pour off through a filter and wash the cylinder and filter with a mixture of 2 parts of absolute alcohol to 3 parts of anhydrous ether, also pouring the wash liquor through the filter. Evaporate the filtrate to a syrupy consistency, dry for an hour at the temperature of boiling water, weigh, ignite, and weigh again. The loss on ignition gives the weight of glycerol.

*Sweet Wines.*—In the case of wines whose extract exceeds 5 grm. per 100 c.c., heat 100 c.c. to boiling in a flask and treat with successive small portions of milk of lime until the wine becomes first darker and then lighter in colour. Cool, add 200 c.c. of 95% alcohol by volume, allow the precipitate to subside, filter, and wash with

95% alcohol. Treat the combined filtrate and washings as directed in the case of dry wines.

The above troublesome processes are likely to remain official until either an exact method is evolved or a simpler method devised which will give results strictly comparable with those obtained by the ether-alcohol method. Windisch (*loc. cit.*, 80-82) gives a complete set of references to the literature of the subject up to 1895. Since then the most important communication has been that of Trillat (*Compt. rend.*, 1902, **135**, 903) who recommends the following simple method, which has some followers in France.

50 c.c. of wine are evaporated to one-third of its volume in a silver or nickel dish at 70°, 5 grm. of animal charcoal are added, and evaporation continued to dryness, when the residue is mixed in a mortar with 5 grm. of quicklime, extracted twice by thorough shaking in a flask with 30 c.c. of dry ethyl acetate (free from alcohol) and the extract filtered, evaporated on the water-bath, and dried in an oven at 60° to constant weight.

Glycerol itself is none too easily extracted by ethyl acetate, and in consequence other workers (Rocques, *Ann. Chim. anal.*, 1905, **10**, 306, and Billon, *Rev. intern. falsif.*, 1906, **19**, 57) have improved on Trillat's method until it is quite as complicated as the ether-alcohol method.

An entirely original method is that of Laborde (*J. Pharm.*, 1895, **6**, 1, 568, and *Ann. Chim. anal.*, 1899, **4**, 76 and 110, and 1905, **10**, 340). It is based on the fact that at 150° to 200° glycerol is quantitatively decomposed by sulphuric acid with the liberation of the whole of the carbon which Laborde weighs as such.

As one of the newer methods may obtain official sanction if experience shows it to be quicker and to give results comparable with those obtained by the present official methods, it has been thought worth while to bring Windisch's list of references up to date, following his classification. In addition to the methods already referred to, the following have been described since 1895:

1. Modifications of the ether-alcohol method.

Fabris, *L'Orosi*, 1897, **20**, 260. Details for manipulation of sweet wines only.

Guglielmetti and Copetti, *Ann. Chim. anal.*, 1904, **9**, 11.

Canonica, *Anal. Soc. Uim. Argentina*, 1918, **6**, 94.

## 2. Oxidation Methods.

Depending on the use of potassium permanganate in acid solution. Mancuso-Lima and Scarlata, *Staz. Sper. Agrar. Ital.*, 1895, **28**, 206.

Depending on the use of chromic acid. Bordas and de Raczkowski, *Compt. rend.*, 1896, **123**, 1021.

## 3. By esterification.

As triacetin. Bottinger, *Chem. Zeit.*, 1897, **21**, 659.

4. By steam-distillation under reduced pressure. Bordas and de Raczkowski, *Compt. rend.*, 1897, **124**, 240.

## 5. By conversion into isopropyl-iodide by Zeisel's method, weighing as silver iodide.

Zeisel and Fanto, *Zeitsch. anal. Chem.*, 1903, **42**, 549.

(See under *Glycerol*, Vol. II.)

**Extract.**—In sweet wines, in which sugar forms an important part of the solids, the extract can be ascertained with fair accuracy from the sp. gr. of the original wine and that of the alcoholic distillate, on the assumptions, not very inaccurate, that a solution of wine solids, containing 10 grm. per 100 c.c., has a sp. gr. of 1.0386 and that the excess gravity over water ( $=1$ ) is proportional to the amount of extract present. If  $s$  be the sp. gr. of the wine, and  $s'$  the sp. gr. of the alcoholic distillate diluted to a volume equal to that of the wine from which it is derived, then the extract,  $x$ , in grams per 100 c.c., is given by:  $x = (s - s') \div 0.00386$ .

If the extract so determined is less than 5% a direct estimation is advisable, as the factor 0.00386 is much less accurate for the other wine solids than for the sugar, which predominates only in wines of relatively high extract-content. To this end 25 c.c. (or 50 c.c. if the extract is below 2.5%) are evaporated to a thick syrup in a 3-in. flat-bottomed platinum dish, transferred without delay from the water-bath to a steam-oven, and after 2 hours, cooled in a desiccator and weighed. If the extract much exceeds 5% it is better to be satisfied with the indirect estimation from the sp. gr., since it is practically impossible to dry such extracts under the above conditions. The direct estimation in any case is purely empirical, the result depending on the size and thickness of the dish and the size, shape and manner of ventilation of the steam-oven; all these are rigidly defined by continental workers.

In Germany no direct estimation is made if the extract exceeds 4%; if it is less than 3% 50 c.c. are evaporated, while if it is between 3 and 4%, so much is evaporated as will leave not more than 1.5 gm.

The official A. O. A. C. directions are to take 50 c.c. for dry wines and 25 c.c. for sweet wines, with the reservation that when the extract exceeds 6% no direct estimation is to be attempted.

In France the official method consists in evaporating 5 c.c. of the wine in a flat-bottomed glass dish (70 mm. diameter and 25 mm. high) *in vacuo*, a dish containing sulphuric acid being placed over that containing the wine. Evaporation is allowed to proceed for 4 days at ordinary temperature after which the residue is weighed.

**Ash.**—The residue from the estimation of extract, or from the evaporation of 25 c.c. of the wine if the extract was not directly determined, is cautiously charred, and repeatedly extracted with small portions of hot water, which are then decanted through a small ashless filter. The filter is then returned to the dish, dried and ashed. When the ash is quite white, the filtrate is added to the contents of the dish, evaporated to dryness, moistened with ammonium carbonate solution, heated to a dull redness, cooled in a desiccator and weighed.

The official A. O. A. C. method is as follows: The residue from 50 c.c. of the wine is charred and burnt, until free from carbon, at a low heat not exceeding dull redness. If a carbon-free ash cannot be obtained in this manner, exhaust the charred mass with hot water, collect the insoluble residue on a filter, burn till the ash is white or nearly so, and then add the filtrate to the ash and evaporate to dryness. Heat to low redness till the ash is white or greyish-white and weigh.

To determine the alkalinity of the water-soluble ash, the ash as obtained above is extracted with successive small portions of hot water until the filtrate amounts to about 60 c.c. The latter is cooled and titrated with N/10 hydrochloric acid, using methyl orange as indicator. The alkalinity is expressed in terms of the number of c.c. of N/10 acid required to neutralise the water-soluble ash from 100 c.c. of the wine.

To determine the alkalinity of the water-insoluble ash, the filter and residue from the soluble portion are ignited in the platinum dish in which the wine was ashed, treated with an excess of N/10 hydro-

chloric acid (usually 10 to 15 c.c.), heated to boiling over an asbestos plate, cooled and titrated with N/10 sodium hydroxide, using methyl-orange as an indicator. The alkalinity is expressed in terms of the number of c.c. of N/10 acid required to neutralise the water-insoluble ash from 100 c.c. of the wine.

**Total Acid.**—25 c.c. of the wine are quickly heated to incipient boiling and quickly titrated with N/2 sodium hydroxide, using litmus paper as indicator. The alkali should be standardised against 25 c.c. of a N/10 solution of an organic acid under the same conditions and with the same indicator. The object of heating is not only to expel carbon dioxide, but to reduce the amphoteric reaction of the wine when nearing neutralisation. If the liquid be heated quickly and only until it begins to boil no appreciable loss of acetic acid will result. The use of N/2 alkali reduces the volume of cold liquid to be added and hastens the titration; the reading is smaller than with N/10 alkali, but is sufficiently large. The acidity is usually calculated as tartaric acid, though in fact free tartaric acid is seldom present in more than traces.

The official A. O. A. C. method is substantially the same as the above but a 0.05% azolitmin solution is used as outside indicator; for wines which are artificially coloured and cannot be titrated in this way, phenolphthalein powder (1 part phenolphthalein mixed with 100 parts of dry, powdered potassium sulphate), may be used as outside indicator.

In France the convention is to calculate the acidity as equal to so many grams of sulphuric acid per 1000 c.c. and it is important to bear this definition of acidity in mind when referring to French standards.

**Volatile Acid.**—50 c.c. of the wine are distilled in a current of steam free from carbon dioxide. A little tannin added to the wine prevents foaming and is preferable to the use of a spray trap. The wine is directly distilled until the volume is reduced to about 25 c.c.; steam is turned on and the flame under the flask so adjusted that the volume remains about 25 c.c. while a total distillate of 200 c.c. is collected. This is titrated with N/10 sodium hydroxide, using phenolphthalein as indicator, and the acidity calculated as acetic acid.

According to Windisch and Roettgen (*Zeitsch. Nahr. Genussm.*, 1911, 22, 155) the total volatile acidity is only obtained in 200 c.c.

of distillate if the wine does not contain more than 0.2%. If the amount much exceeds this, the wine should be diluted and 50 c.c. of the diluted sample used.

**Fixed Acid.**—This is estimated by difference. Since the total acid was reckoned as tartaric and the volatile as acetic acid, the fixed acid in terms of tartaric acid is found by subtracting 1.25 times the volatile acid from the total acid.

It is not permissible to estimate the fixed acids directly by evaporating to dryness, with subsequent titration of the redissolved residue, since fixed acids may be destroyed during the last stages of the evaporation. On the other hand, Windisch (*Zeitsch. Nahr. Genussm.*, 1905, 9, 70) has shown that the above-described method for the estimation of volatile acids is not quite satisfactory on account of the partial volatility of lactic acid, which may be the chief acid present, and he suggests the following method. 25 c.c. of the wine are evaporated to about 3 c.c., 25 c.c. of hot water added, and the liquid again evaporated to 3 c.c., and this process repeated once more. The residue is diluted and titrated with standard alkali. From the result is calculated the fixed acid and from this and the total acid the amount of volatile acid may be obtained indirectly.

**Reducing sugar** is estimated with Fehling's solution. 200 c.c. of wine are neutralised with sodium hydroxide and evaporated to about 50 c.c., cooled, transferred to a 200 c.c. flask and diluted to about 160 c.c. Basic lead acetate solution<sup>1</sup> (20 c.c.) is added and the contents of the flask made up to the 200 c.c. mark with water. The mixture is shaken and filtered. To 100 c.c. of the filtrate, 10 c.c. of a saturated solution of sodium sulphate are added, the mixture shaken and filtered. The filtrate, a volume of 11 c.c. of which corresponds to 10 c.c. of wine, serves for the estimation of reducing sugar.

The official A. O. A. C. method differs from the above in that neutral lead acetate solution is used for clarification instead of the basic salt, and the filtrate is freed from lead with dry potassium oxalate; with this procedure of course, the volume of the filtrate corresponds to that of the wine. The reducing sugar content of the filtrate (usually expressed as invert sugar), is deter-

<sup>1</sup> Prepared by boiling for half an hour 430 grm. lead acetate, 130 grm. litharge and 1000 c.c. water, allowing to cool and settle, and subsequently diluting the clear liquid to 1.25 sp. gr. with recently boiled water.

mined either gravimetrically or volumetrically as described in the "Sugars" section of this work.

*Note.*—In order that results by the gravimetric method may be calculated by aid of the published tables, it is necessary that the amount of copper or oxide to be weighed shall fall within certain limits. If the sugar-content does not exceed 1 grm. per 100 c.c. the above quantities will give a convenient precipitate. If the proportion of sugar is greater than 1% a smaller quantity of wine should be taken in the first instance. The sugar-content is as a rule not very far from  $x - 2$ , where  $x$  represents the grams of extract per 100 c.c. If therefore a wine shows 4% extract, it will probably contain about 2% of sugar, and 100 c.c. is then diluted with an equal volume of water, evaporated down to about 50 c.c., cooled and made up to 200 c.c. with lead acetate, etc., as already described.

**Cane Sugar.**—50 c.c. of the clarified solution taken for the estimation of reducing sugars are exactly neutralised with hydrochloric acid, 5 c.c. of 1% hydrochloric acid added, and the whole heated for half an hour on the water-bath. The liquid is exactly neutralised, evaporated somewhat, made slightly alkaline with sodium carbonate and filtered into a 50 c.c. flask, the filter being washed until the flask is full to the mark. The reducing power of this filtrate is now determined by means of Fehling's solution, and the result calculated as invert sugar. As 95 parts of cane sugar yield 100 parts of invert sugar on hydrolysis, the amount of cane sugar in 100 c.c. of the wine is given by  $x = 0.95 (b - a)$ , where  $a$  is the amount of reducing sugar, expressed as invert, in 100 c.c. of the original wine, and  $b$  the amount found after inversion.

The official A. O. A. C. method differs from the above in that the clarification of the original solution is effected with neutral lead acetate, the excess of lead being removed with potassium oxalate, and the inversion is carried out as described in the "Sugars" section (page 360).

**Polarisation.**—The polarimeter may give useful information concerning a sample of wine, especially one suspected of sophistication. The following scheme may be found useful. It remains nearly in the words of a Bulletin of the A. O. A. C. but has been slightly altered in conformity with the German decree (*Veröffentl. d. kaiserl. Gesundheitsamtes*, 1896, 20, 557) on which it is based.

All results are to be stated as the polarisation of the undiluted wine in a 200 mm. tube. The Schmidt and Haensch half-shadow saccharimeter is to be used, and the results expressed in terms of the sugar scale of this instrument. If any other instrument be used, or if it be

desirable to convert to angular rotation, the factors given on page 372 are to be used.

*White Wines.*—60 c.c. of wine are neutralised, evaporated to one-third, made up again to 60 c.c., treated with 3 c.c. of basic lead acetate solution and filtered. 31.5 c.c. of the filtrate are treated with 1.5 c.c. of a saturated solution of sodium carbonate, filtered, and polarised. This gives a dilution of 10 to 11, which must be considered in the calculation, and the polarimeter reading must accordingly be increased one-tenth.

*Red Wines.*—60 c.c. of wine are neutralised, evaporated to one-third, made up again to 60 c.c., decolourised with 6 c.c. of basic lead acetate solution and filtered. To 33 c.c. of the filtrate 3 c.c. of a saturated solution of sodium carbonate are added, the mixture filtered, and the filtrate polarised. The dilution in this case is 5 to 6, and the polarimeter reading must accordingly be increased one-fifth.

*Sweet Wines, Before Inversion.*—100 c.c. are neutralised, evaporated to one-third, made up again to 100 c.c., decolourised with 2 c.c. of basic lead acetate solution and filtered after the addition of 8 c.c. of water. 0.5 c.c. of a saturated solution of sodium carbonate and 4.5 c.c. of water are added to 55 c.c. of the filtrate, and the liquid mixed, filtered, and polarised. The polarimeter reading is multiplied by 1.2.

*After Inversion.*—33 c.c. of the filtrate from the lead acetate clarification in (1) are placed in a flask with 3 c.c. strong hydrochloric acid. After thorough admixture of its contents the flask is placed in water and heated until a thermometer, placed in the flask with the bulb as near the center of the liquid as possible, marks 68°, consuming about 15 minutes in the heating. It is then removed, cooled quickly to room temperature, filtered, and polarised, the temperature being noted. The polarimeter reading is multiplied by 1.2.

*After Fermentation.*—50 c.c. of wine are de-alcoholised and made up to the original volume with water, and mixed in a small flask with well-washed beer yeast and kept at 30° until fermentation has ceased, which requires from 2 to 3 days. The liquid is then washed into a 100 c.c. flask, and a few drops of a solution of acid mercuric nitrate and then basic lead acetate solution, followed by sodium carbonate, added. The flask is filled to the mark with water, shaken, and the solution filtered and polarised.

(1) *The Wine Shows No Rotation*

This may be due to the absence of any rotatory substance or to the simultaneous presence of dextrorotatory and lævorotatory sugars.

(a) *The Wine is Inverted.*—A lævo-rotation shows that the sample contained cane sugar.

(b) *The Wine is Fermented.*—A dextro-rotation shows that both lævorotatory sugar and the unfermentable constituents of commercial dextrose were present.

If no change takes place in either (a) or (b) in the rotation it proves the absence of unfermented cane sugar, the unfermentable constituents of commercial dextrose, and of lævorotatory sugar.

(2) *The Wine Rotates to the Right*

This may be caused by unfermented cane sugar, commercial dextrose, or both.

*The Wine is Inverted.*

(a<sub>1</sub>) *It Rotates to the Left after Inversion.*—Unfermented cane sugar was present.

(a<sub>2</sub>) *It Rotates More than 2.3° to the Right.*—The unfermentable constituents of commercial dextrose are present.

(a<sub>3</sub>) *It Rotates Less than 2.3° and More than 0.9° to the Right.*—It is in this case treated as follows:

210 c.c. of the wine are evaporated to about one-third volume to expel alcohol, cooled, diluted with water to the original volume, and fermented with 2 grm. of pressed yeast. The fermented liquid is evaporated in a porcelain dish to a thin syrup with a little sand and a few drops of a 20% solution of potassium acetate. To the residue 200 c.c. of 90% alcohol are added, with constant stirring. The alcoholic solution is filtered into a flask, and the alcohol removed by distillation until about 5 c.c. remain. The residue is mixed with washed bone-black, filtered into a graduated cylinder, and washed until the filtrate amounts to 30 c.c. If the filtrate shows a dextro-rotation of more than 1.5° it indicates the presence of the unfermentable constituents of commercial dextrose.

(3) *The Wine Rotates to the Left*

It contains unfermented lævorotatory sugar, derived either from the must or from the inversion of added cane sugar. It may, how-

ever, also contain unfermented cane sugar and the unfermentable constituents of commercial dextrose.

(a) The wine is fermented according to the process already described.

(a<sub>1</sub>) It polarises  $-3^{\circ}$  after fermentation. It contained only lævorotatory sugar.

(a<sub>2</sub>) It rotates to the right. It contained both lævorotatory sugar and the unfermentable constituents of commercial dextrose.

(b) The wine is inverted according to the process already described.

(b<sub>1</sub>) It is more strongly lævorotatory after inversion. It contained both lævorotatory sugar and unfermented cane sugar.

**Potassium Sulphate.**—50 c.c. of the original wine are acidified with hydrochloric acid, precipitated hot with barium chloride and the sulphate found calculated as potassium sulphate.

**Sulphurous Acid.**—The sulphuring of casks is a common and not improper practice, but the presence of a considerable amount of acid in wine indicates that sulphites have been added as preservative. Some of this sulphurous acid is combined with aldehyde, and since in this form it is said to be less objectionable (Marischler, *Wien. klin. Wochenschr.*, 1896, 31), it is usual to make two estimations, one of total sulphurous acid, the other of sulphurous acid not in organic combination, which latter is in contradistinction described as "free" sulphurous acid.

**"Free" Sulphurous Acid.**—To 50 c.c. of the wine, contained in a flask, a little sodium carbonate is added and then excess of dilute sulphuric acid. The flask is thus filled with carbon dioxide and the sulphurous acid can be titrated fairly accurately with N/50 iodine solution and starch.

**Total Sulphurous Acid.**—50 c.c. of the wine are mixed in a flask with 25 c.c. of normal sodium hydroxide to liberate the sulphurous acid from its combination with aldehyde. After the mixture has stood 15 minutes with occasional shaking, 10 c.c. of dilute (1:3) sulphuric acid are added, and the total sulphurous acid quickly titrated with N/50 iodine solution.

The above method is sufficiently accurate for most purposes, but more exact results may be obtained by distillation methods. The official A. O. A. C. method, which is substantially the same as the official French method, is as follows:

Distil 20 to 100 gramm. of the sample (adding recently boiled water if necessary) in a current of carbon dioxide after the addition of about 5 c.c. of a 20% glacial phosphoric acid solution, until 150 c.c. have passed over. Collect the distillate in about 100 c.c. of nearly saturated bromine water, allowing the end of the condenser to dip below the surface. The method and apparatus may be simplified without material loss of accuracy by omitting the current of carbon dioxide, adding 10 c.c. of phosphoric acid instead of 5 c.c. and dropping into the distillation flask, immediately before attaching the condenser, a piece of sodium bicarbonate weighing not more than 1 gramm. The carbon dioxide liberated is not sufficient to expel the air entirely from the apparatus, but will prevent oxidation to a large extent. When the distillation is finished boil off the excess of bromine, dilute the solution to about 250 c.c., add 5 c.c. of hydrochloric acid (1:3), heat to boiling and precipitate the sulphuric acid with 10% barium chloride solution and weigh as barium sulphate.

**Tannin.**—The following approximate method, due to Nessler and Barth (*Zeitsch. anal. Chem.*, 1883, **22**, 595) has many followers on the continent. 12 c.c. of wine are shaken with 30 c.c. of 96% alcohol and filtered. 35 c.c. of the filtrate, corresponding to 10 c.c. of wine, are evaporated to 6 c.c. and transferred to a measuring tube of prescribed dimensions, the volume brought up to 10 c.c. by addition of water, 1 c.c. of 40% sodium acetate added and finally 1 or 2 drops of 10% ferric chloride. The whole is then shaken and, after 24 hours, the volume of the precipitate is read off. This volume in c.c. multiplied by 0.033 is said to give the approximate percentage of tannin in the wine. The lower part of the measuring tube is 0.8 cm. wide and shows tenths of a c.c., and is long enough to hold about 4 c.c.; the upper part is 1.8 cm. wide and has marks at 10, 11, 20 and 22 c.c. With red wines, it is usual to add 11 c.c. of water immediately after the addition of the ferric chloride and before shaking.

For more exact work, Neubauer's modification of Löwenthal's method (see "Tannins," Vol. V) is most often employed. 10 c.c. of wine is a convenient quantity to take for the process.

**Salicylic Acid.**—This properly belongs to another section of this work. Since, however, genuine unadulterated wine may give the reactions of salicylic acid, if large enough quantities are taken for the test, the German official directions for carrying out the test are given here. It is said that few or no genuine wines will give the character-

istic reactions of salicylic acid when the test is conducted with these quantities, whereas added salicylic acid will be infallibly detected, since a quantity undetectable in this way would not appreciably increase the keeping properties of wine.

50 c.c. of wine are shaken (not too vigorously, lest an emulsion form) with 50 c.c. of a mixture in equal proportions of ether and petroleum spirit. The ethereal layer is separated, filtered and evaporated, and the residue tested with very dilute ferric chloride solution. The tannin is almost insoluble in the mixture of ether and petroleum spirit, but if a black or dark brown colour result on the addition of ferric chloride, a drop of hydrochloric acid is added and the extraction with the solvent repeated.

**Saccharin.**—In the absence of salicylic acid, any of the methods described under *Saccharin* in a later volume of this work may be applied. Methods depending on the conversion of saccharin into salicylic acid are clearly not applicable when salicylic acid is present.

The following method, due originally to Herzfeld and Reischauer (*Deutsche Zuckerind.*, 1886, 124), was especially recommended by Allen (*Analyst*, 1888, 13, 105).

100 c.c. of wine are mixed with coarse sand and evaporated on the water-bath. The residue is treated with 1 or 2 c.c. of 30% phosphoric acid and repeatedly extracted with a moderately warm mixture, in equal proportions, of ether and petroleum spirit. The successive extracts, which should amount in all to 200 c.c. or more, are filtered through asbestos and the greater part of the solvent removed by distillation. The concentrated extract is poured into a basin, the remainder of the solvent evaporated, and the residue taken up with dilute sodium carbonate solution, filtered into a platinum dish and evaporated to dryness.

The residue is mixed with 4 or 5 times its bulk of dry sodium carbonate and added in small portions to fused nitre. The fused mass is dissolved in water, acidified with hydrochloric acid and treated with barium chloride. Each gram. of barium sulphate corresponds to 0.786 gram. saccharin.

**Boric Acid.**—Methods of estimating this need no description here, but it may be pointed out that merely qualitative tests are valueless, since it is now known that boric acid is a normal constituent of wines.

**Fluorides.**—For the estimation of fluorides in wine, Treadwell and Koch (*Zeitsch. anal. Chem.*, 1904, 43, 469) recommend the following

modification of Rose's method. 100 c.c. of the wine are rendered feebly alkaline with sodium hydroxide, and silver nitrate is added as long as it produces a precipitate. The mixture is then made up to 250 c.c., filtered, and 200 c.c. of the filtrate treated with an excess of sodium chloride, and made up to 250 c.c. After 24 hours, 175 c.c. of the clear liquid are decanted and treated with 3 or 4 c.c. of 2N sodium carbonate solution, and then boiled for 5 minutes with a large excess of calcium chloride. The precipitate is collected on a filter, washed with hot water, dried and heated to dull redness for 15 minutes. When cool, 3 c.c. of 9% acetic acid are added to the contents of the crucible and the whole digested on the water-bath for half an hour, after which the mixture is evaporated to dryness. Two drops of 9% acetic acid are added, and the residue in the crucible repeatedly extracted with small portions of hot water, which are then passed through a small filter. This is subsequently washed, dried, and ignited, the ash returned to the crucible and the latter ignited and weighed. Extraction with acetic acid should be repeated until two weighings, differing by less than 0.5 mg., are obtained. 1.6 mg. are added to the weight for each 100 c.c. of wash-water used.

Occasionally, but rarely, chlorine, lime, magnesia, and potassium oxide are estimated in wines. The methods for their estimation need no description here.

**Tartaric Acid.**—For the estimation of total tartaric acid, free tartaric acid, potassium hydrogen tartrate, and calcium tartrate, Halenke and Möslinger (*Zeitsch. anal. Chem.*, 1895, **34**, 279) have worked out an exact scheme of analysis, which is described very fully by Windisch (*loc. cit.*, 120). These determinations are apparently considered of importance by German officials, since the methods of Halenke and Möslinger are set out in some detail in the Imperial decree to which reference has already been made. The results will have more significance when a larger number of genuine wines have been examined by the accurate methods to which reference has been made. Most of the published statistics are based on older and less exact methods of analysis.

The official A. O. A. C. method is as follows: Neutralise 100 c.c. of the wine with N/1 sodium hydroxide solution, calculating from the total acidity the number of c.c. of N/1 alkali necessary for the neutralisation. If the volume of the solution is increased more

than 10% by the addition of the alkali, evaporate to approximately 100 c.c. Add to the neutralised solution 0.075 gm. of tartaric acid for each c.c. of N/1 alkali added and, after the tartaric acid has dissolved, add 2 c.c. of glacial acetic acid and 15 gm. of potassium chloride. After the potassium chloride has dissolved add 15 c.c. of 95% alcohol by volume, stir vigorously until the potassium hydrogen tartrate begins to precipitate and then leave the mixture in an ice box for at least 15 hours. Decant the liquid from the separated potassium hydrogen tartrate on a Gooch crucible prepared with a very thin film of asbestos, or on a filter paper in a Büchner funnel. Wash the precipitate and filter three times with a few c.c. of a mixture of 15 gm. of potassium chloride, 20 c.c. of 95% alcohol by volume and 100 c.c. of water, using not more than 20 c.c. of the wash solution in all. Transfer the asbestos or paper and precipitate to the beaker in which the precipitation was made, wash out the Gooch crucible or Büchner funnel with hot water using about 50 c.c. in all, heat to boiling and titrate the hot solution with N/10 sodium hydroxide solution, using phenolphthalein as an indicator. Increase the number of c.c. of N/10 alkali required by 1.5 c.c. to allow for the solubility of the precipitate. 1 c.c. of N/10 alkali is equivalent, under these conditions, to 0.015 gm. of tartaric acid. Subtract the amount of tartaric acid added from this result to obtain the weight of total tartaric acid per 100 c.c. of the wine.

The free tartaric acid and cream of tartar are calculated in the following manner:

Let  $A$  = total tartaric acid in 100 c.c. of wine, divided by 0.015;  
 $B$  = total alkalinity of the ash (sum of  $C$  and  $D$ );  $C$  = alkalinity of water-soluble ash; and  $D$  = alkalinity of water-insoluble ash.  
 Then:

- (1) If  $A$  is greater than  $B$ ,  
     Cream of tartar =  $0.0188 \times C$ ; and  
     Free tartaric acid =  $0.015 \times (A - B)$ .
- (2) If  $A$  equals  $B$  or is smaller than  $B$  but greater than  $C$ ,  
     Cream of tartar =  $0.0188 \times C$ ; and  
     Free tartaric acid = 0.
- (3) If  $A$  is smaller than  $C$ ,  
     Cream of tartar =  $0.0188 \times A$ ; and  
     Free tartaric acid = 0.

**Foreign Colouring Matters.**—Wines should always be examined for the presence of coal-tar colours, and tested as to their behaviour with lead acetate. Even white wines may have received an addition of caramel or of a coal-tar colour specially prepared as a caramel substitute. Some cleaned wool, mordanted with alum and sodium acetate, should be boiled with the wine and any precipitated colour examined by the usual reagents (see Vol. V, and Green, *J. Soc. Dyers and Col.*, 1905, 21, 236). It is usually sufficient to establish the presence of such colours, however, and not necessary to identify them.

The following tests have been specially recommended for the detection of foreign colouring matters in wine.

**Lead Acetate Test.**—5 c.c. of basic lead acetate solution are added to 20 c.c. of wine. If the resulting precipitate is red-violet in colour, the fact is strong evidence of the presence of the colouring matter of poke berries (*Phytolacca decandra*). Bilberry juice gives a blue precipitate and mallow and elderberry juice a green one, but these colours are much less characteristic than that given by poke. Genuine wines may give a grey, blue-grey, blue-green or green precipitate, but never a red-violet one. Another 5 c.c. of lead acetate is added and the liquid warmed and filtered. If the filtrate is red, rosaniline may be suspected, but some genuine dark red wines are only with difficulty decolourised by basic lead acetate. If amyl alcohol, shaken with the filtrate, assumes a red colour the presence of artificial colouring matters may be inferred with certainty.

**Wool Test.**—White wool, mordanted with alum and sodium acetate, is boiled with the wine to which 10% of its bulk of 10% potassium sulphate has been added. Genuine wines may impart a red colour to the wool which remains after washing, but this colour is much less intense than that given by minute traces of coal-tar dyes and may be distinguished by its turning a dirty greenish-white on treatment with ammonium hydroxide. If the colour is of coal-tar origin, it will remain unchanged or change to a yellowish tint, which reverts to red on washing out the ammonia.

**Cazeneuve's Mercuric Oxide Test.**—10 c.c. of wine are shaken with 0.2 grm. yellow mercuric oxide for at least a minute, and when the oxide has completely settled the liquid is filtered. Several thicknesses of paper are sometimes necessary and if a clear filtrate cannot be obtained in this way, the experiment should be repeated and the

mixture heated to boiling before shaking. A clear but coloured filtrate indicates the presence of coal-tar colours, but a colourless one is no proof of their absence, since many, including the rosanilines, are absorbed by mercuric oxide. The test serves, however, to detect acid fuchsin, Bordeaux red, and other colours which escape the test with basic lead acetate.

**Shaking with Ether before and after Supersaturation with Ammonium Hydroxide.**—To 100 c.c. of wine, 5 c.c. of ammonium hydroxide are added, and the mixture shaken with 30 c.c. of ether. Another 10 c.c. of wine are shaken with ether without the addition of ammonia. From each ethereal layer, 20 c.c. are withdrawn with a pipette and allowed to evaporate in a basin containing a thread of wool about 2 in. long. If the wool from the experiment in which the ammonia was used is dyed red, the presence of coal-tar colours may be inferred. With genuine wines the wool from the experiment in which ammonia was used remains perfectly white, whilst that from the experiment without ammonia usually acquires a brownish tint. The test serves for the detection of rosaniline, safranin, and chrysoidin, but acid fuchsin and many other colours which may be present are not detected by it.

**Shaking with Amyl Alcohol.**—This test is best performed in triplicate: (a) on the original wine; (b) on the wine made acid with sulphuric acid, and (c) on the wine made alkaline with ammonia. 100 c.c. of wine and 30 c.c. of amyl alcohol are convenient quantities.

(a) If the amyl alcohol is coloured red the presence of artificial colouring matter is not necessarily to be inferred, since many high-coloured young wines yield red colouring matter to amyl alcohol. If, on the addition of a few drops of ammonia, the colour remains unchanged, the presence of coal-tar colours is tolerably certain, since the red colour of genuine wines is changed to blue or green on such treatment.

(b) The amyl alcohol extract from acidified red wines is generally red, but any artificial colour is concentrated by the treatment and separated from some other matters which may mask its reactions. The amyl alcohol is shaken with water and the aqueous solution tested with ammonia or submitted to the wool test.

(c) If the amyl alcohol extract from the ammoniacal wine is red, the presence of coal-tar colours may be safely inferred. If it is colourless the experiment should be repeated, less ammonia being

used, since in presence of a large excess (above 3%) of ammonia the amyl alcohol may remain colourless even when coal-tar colours are present.

The detection of *caramel* in wine is less important now than it was some years ago, since tar colours have been specially prepared to replace it, and the necessary quantity of these tar colours is so small that their expense is negligible, and the sophisticator probably thinks them less easy of detection. In point of fact, the time-honoured tests for caramel fail to detect modern preparations sold under this name. This is not surprising, since the caramel of to-day is a widely different product from the burnt sugar of 20 years ago; it contains a notable proportion of amino-compounds and is chemically very different from earlier preparations, in the published analyses of which nitrogen was never recorded. The white-of-egg test of Carles (*J. Pharm. Chim.*, 1875, **22**, 177) remains in all the text-books, although a liquid coloured by a modern preparation of caramel loses quite as much colour as many genuine wines on treatment with white of egg. Schidrowitz (*J. Soc. Chem. Ind.*, 1902, **21**, 816) has doubly discredited Amthor's paraldehyde test (*Zeitsch. anal. Chem.*, 1885, **24**, 30), which may discover caramel where there is none and fail to discover it when actually present.

In the process of caramelising sugar, hydroxymethylfurfural is formed; this compound gives characteristic colours with certain phenols in presence of strong acids and these colour reactions have been utilised by Jagerschmid (*Zeitsch. Nahr. Genussm.*, 1909, **17**, 269) and Schenk (*Apoth. Zeit.*, 1914, **29**, 202) for detecting caramel in wine, brandy, etc. According to Schenk, the wine, if acid, is neutralised, concentrated to a small volume and extracted with ether. The ethereal extract is treated with a few drops of a 5% solution of phenol in ether and allowed to evaporate. The residue is mixed with about 5 c.c. of sulphuric acid, when an orange-yellow colouration indicates caramel. If naphthol is used instead of phenol a violet colour is obtained, whilst if resorcinol and hydrochloric acid are used, the colour is cherry red (compare Fiehe's test for invert sugar in honey described in the "Sugars" section, p. 480).

The foregoing tests have many years' successful application to recommend them. No single one of them will carry the analyst very far, but if they are all applied, few artificially coloured wines will escape detection. Those few will be coloured with bilberry juice

or similar fruit juice, and the methods described for the detection of bilberry juice are so troublesome, and withal so uncertain, that they are scarcely worth description here.

New methods for the detection of foreign colouring matters, especially of magenta, in wines are described each year, and occasionally the claim is made for a new test that it enables the analyst by a single operation to decide whether a sample has been artificially coloured or not. The continued use of the older, more troublesome methods by experienced analysts must be taken as evidence that the comprehensiveness of the new tests yet lacks proof.

One of the best of recent suggestions is that of Jean and Frabot (*Ann. chim. anal.*, 1907, **12**, 52, and *Bull. Soc. Chim.*, 1907, **1**, 748). Extending some experiments of Trillat, these authors find that all genuine wines yield a colourless filtrate when treated as follows: 50 c.c. of the wine are warmed on the water-bath with 1 c.c. of formalin and 4 c.c. of hydrochloric acid. When a precipitate has formed, an excess of ammonia is added and the heating continued till all the free ammonia has been expelled. The liquid is then cooled and filtered. Artificially coloured wines when treated in this way are said to yield a coloured filtrate. It is useful to know that all genuine wines which have been tested in this way yield colourless filtrates, but it would be unwise to regard such a colourless filtrate as proof that no colour other than that natural to the wine was present.

The following method, due to Dupré (*J. Chem. Soc.*, 1880, **37**, 572), has been useful to many. The best colourless commercial gelatin is dissolved in 10 parts of boiling water, and the solution poured into a soup-plate or other flat vessel. When cold and thoroughly set, a cube about  $\frac{3}{4}$  in. on the side is cut from the jelly by means of a sharp knife and placed in the sample of wine to be tested. After standing 24 hours, the cube is removed, washed a little with cold water, and a central slice cut out of it in a direction parallel to one of the sides. On examining this section, it will be found, in the case of a pure wine, that the colouring matter has penetrated but a very little way into the jelly (perhaps  $\frac{1}{16}$  in.), whereas the great majority of foreign colouring matters will have penetrated to the very centre of the cube.

Of a large number of colouring matters only that of *alkanet-root* resembles the "œnolin" of pure wine in the slow rate at which it

diffuses into the jelly. Hence, if coloration of the interior of the jelly is not observed, alkanet is the only foreign colouring agent likely to be present. It may be distinguished by its absorption-spectrum, which, at a certain concentration of the acidified solution, shows three distinct absorption-bands between the sodium line and the blue strontium line, and nearly equidistant from these lines and from each other. Ammonia changes the colouring matter of alkanet to blue, and reduces the absorption-bands to two, one coincident with the *D* line and the other less refrangible than that. Both acid and alkaline solutions produce a general absorption of the violet end of the spectrum, and in moderately concentrated solutions only the red is transmitted.

The colouring matter of pure red wine produces a general absorption in all parts of the spectrum except the red, but generally no distinct absorption-band. The red colour is changed to greenish-brown on addition of ammonia, and the liquid then shows an indistinct absorption-band in the orange-yellow region.

If the colouration of the cube of jelly points to the presence of a foreign colouring matter, the nature of this may frequently be ascertained, if desired. As a rule, the slice of jelly shows the colour proper to the added substance much more clearly than did the wine itself, and a difference between the two colours is a strong indication of the presence of a foreign matter. *Indigo* and *logwood* may thus be readily discovered. The absorption-spectrum exhibited by the slice will serve for the detection of *rosaniline*, *cochineal*, *beet-root*, *red-cabbage*, *litmus*, etc., and further information may be gained by placing the slice in dilute ammonia. Thus treated, a slice coloured with *rosaniline* becomes colourless; with *red cabbage*, dark green; with *cochineal*, purple, and with *logwood*, brown. This last reaction is, however, frequently produced in the absence of logwood. When present, the slice will be coloured brown or yellow to a considerable depth before it is treated with ammonia.

Operating in the above manner, Dupré found that an addition of foreign colouring matter equal to 10% of the total intensity of the colour of the wine could usually be readily detected, and in no case could 20% be overlooked. In the case of logwood 5% could be recognised, and as little as 1% of *rosaniline* could be found. In making the tests it is desirable to compare the sample with a pure wine of the same kind.

**Physico-chemical Analysis of Wines.**—The time has not yet come for the description, in a general work like this, of the physico-chemical methods of P. Dutoit and M. Duboux. Nothing less than a detailed description, occupying much space, could serve any useful purpose, and this would scarcely be justified, seeing that the methods are not yet used in this country, so far as the writer is aware, whilst they are ignored by all German writers, *e. g.*, by the author of the section on Wines in the most recent edition of Lunge's *Technical Methods of Analysis*.

However, the application by Dutoit and Duboux of their methods to the investigation of wine dates back at least to 1908 and is of general interest, if only for its originality. It is quite probable that the methods may come into general use in laboratories where a large amount of routine work has to be conducted. Such work on wine is not often undertaken in Great Britain, but those who refer to this book have a right to expect at least a reference to work which may become important in the future. Fortunately, the authors have published a small book,<sup>1</sup> which makes it unnecessary to give references to their original papers, some of which appeared in Swiss periodicals, not readily accessible.

Briefly, their methods consist in titrating the wine with appropriate reagents and taking successive conductivity measurements, the results being plotted on a system of rectangular coordinates. In this way, curves are obtained which show distinctly the saturation points. Not only can chlorides, sulphates, phosphates, lime, total alkalinity, total acidity, ash and ammonia be thus estimated, but also tartaric, malic and succinic acids.

The book referred to embodies their work up to 1912, since when only two papers have been published.

### SIGNIFICANCE OF RESULTS OF WINE ANALYSIS

The chemist who needs to refer to a general work of this kind for analytical methods will presumably only seek to know how he may distinguish genuine wine from adulterated beverages. The significance of the results to the owner or intending purchaser of wine of undoubted genuineness but doubtful capacity for improvement on keeping cannot be dealt with here; experience of the wines of a particular district and the possession of a trained palate are indis-

<sup>1</sup> *L'Analyse des Vins par Volumetrie Physico-Chimique*. Lausanne, Rouge et Cie.

pensable to the formation of a sound judgment of the future behaviour of a wine.

The following notes assume that the analyst is concerned only in deciding as to the genuineness or otherwise of a sample. Thus the alcoholic content is described as of small significance, which from this standpoint is true, but to a wine expert a difference of 2% may suggest a great deal as to the relative stability of two wines.

The standards most respected in France and Germany are applicable only to wines which should be the product of fermentation of normal grape-musts, without concentration of these musts or addition of alcohol, sugar or other substance. They are occasionally subjected to criticism even in the countries of their origin, and it would be unfair to apply them to the wines of other countries. The standards which have been proposed for certain sweet wines are of still narrower application, and there are insufficient data available concerning the wines of Spain and Portugal to justify any standards for the admittedly fortified wines of those countries. Except when otherwise stated, the following notes refer only to wines which, if genuine, are the undiluted product of the fermentation of pure grape-musts, and the word "genuine" when used is to be understood in this sense.

**Specific Gravity.**—This is of small significance in judging a wine, and the main purpose of ascertaining it is the estimation of extract by the indirect method. The sp. gr. of wines derived by natural fermentation from the juice of the grape, without concentration or addition of any kind, is never far from unity, seldom less than 0.99 and, according to certain French authorities, never less than 0.985. In wines which may properly be derived from concentrated musts or to which the addition of alcohol is a recognised practice, the variations in sp. gr. may be very great.

**Alcohol.**—The alcohol-content of genuine wines usually lies between 5 and 10 grm. per 100 c.c., but numbers as low as 2.1 and as high as 12.2 have been recorded. Alcohol in excess of 14.5 grm. per 100 c.c. would be certain evidence of added spirit, but it must be remembered that even in Germany such addition is permitted by law, provided it does not exceed 0.8 grm. per 100 c.c. of wine. Since the alcohol-content of genuine wine may vary so widely, the number is of small value in determining whether a sample has been diluted with water.

**Glycerol.**—The proportion of glycerol usually lies between 0.4 and 1% but may be as low as 0.16 or as high as 1.4%.

**Alcohol-glycerol Ratio.**—German chemists attach more importance to this number than to the absolute percentages of alcohol or glycerol, and this is reasonable, but the standards set up some years ago require amendment even for German wines. It was formerly supposed that the ratio of alcohol to glycerol in genuine wines always lay inside the limits 100:7 and 100:14. A wine which showed a higher ratio of alcohol to glycerol than 100:7 was held to have been fortified by addition of spirit, while if the ratio fell below 100:14, addition of glycerol was suspected. It is now known that in genuine Rhine wines the alcohol-glycerol ratio may exceed 100:6 or fall below 100:19. If 100:5 and 100:20 be taken as the limits, few genuine European wines will be excluded, but Bigelow has pointed out that the average alcohol-glycerol ratio for American wines is about 100:6 and in his table are included wines in which it is as high as 100:2.

**Extract.**—The percentage of sugar varies within wide limits, and plastered wines may contain notable quantities of potassium sulphate. Apart from these two constituents, the percentage of solids in solution in young wines is fairly constant and has been ascertained to be never less than a certain amount. Unfortunately, French chemists are not content with recording the extract less sugar and potassium sulphate, but, since these latter are normally present in small amount, they define "reduced extract" as  $x - (S - 0.1) - (K - 0.1)$ , where  $x$ ,  $S$  and  $K$  represent the percentage of extract, sugar and potassium sulphate in the wine. For the purpose of comparison with arbitrary standards the simpler formula  $x - S - K$  would serve equally well, but in this and the following paragraphs the expression "reduced extract" is used in the French sense. The reduced extract of genuine white wine of continental origin is seldom less than 1.6 grm. per 100 c.c., that of red wine seldom less than 1.8 grm. The amount of extract decreases with age, but seldom falls below 1.5 grm. Bigelow quoting M. Curtis, of San Francisco, says that American red wine is to be viewed with suspicion if it contain less than 2.4 or more than 3.3 grm. reduced extract per 100 c.c. For American white wine he places the limits at 1.5 and 2.4 grms.

**Alcohol-extract Ratio.**—In France more importance is attached to this number than to the ratio of alcohol to glycerol. It is said that

for genuine red wines the ratio never exceeds 4.5, whilst for white wines it may be higher but is never in excess of 6.5. Higher values are to be taken as proof of added alcohol. This test is more severe when the French method of determining extract is used, since by that method glycerol is largely driven off, and a lower number obtained for the extract.

**Ash.**—The ash-content of wines usually lies between 0.2 and 0.3% but genuine wines have been known to contain as little as 0.11 and as much as 0.44%. A smaller amount than 0.14% would justify suspicion, but it is less easy to fix an upper limit, though it may be fairly said that 0.35% is rarely exceeded. The ash follows the reduced extract to some extent, and is higher for red wines. In attempting to draw conclusions from the amount of ash it is well to deduct from this the percentage of potassium sulphate found less 0.1.

**Total Acid.**—The total acid, calculated as tartaric acid, is seldom less than 0.4 or more than 1.5%. In France the total acid is calculated as sulphuric acid, and it is held that the sum of the alcohol (expressed as c.c. per 100 c.c.) and the acid (calculated as grams of sulphuric acid per 1000 c.c.) is never less than 12.5 for a genuine wine. A lower value is held to be evidence of dilution with water. This is perhaps the most frankly empirical standard which has been applied to wines, but it has behind it the experience of a whole generation of French chemists and may presumably be applied with confidence to wines purporting to be of French origin. When the alcohol-extract ratio exceeds 4.5 for red wines or 6.5 for white wines, the "natural" percentage of alcohol is substituted for that actually found. For example, if a red wine contains 12 grm. alcohol and 1.5 grm. reduced extract per 100 c.c., it is obvious that alcohol has been added. This added alcohol must not be taken into account in applying the test for added water. Instead, the extract, 1.5, is multiplied by 4.5 to give the "natural percentage" by weight of alcohol, and then divided by 0.8 to obtain the percentage by volume. To the number so obtained, in this case 8.5, the acidity in grm. per 1000 c.c. is added, and if the sum is less than 12.5 it may be taken as evidence that both water and alcohol have been added.

**Volatile Acid.**—The volatile acid, calculated as acetic acid, is usually below 0.08% and wine containing much more than 0.15% would be condemned not as fraudulent, but as unsound. As has been said already, considerable experience is necessary in forming

judgments of soundness. Thus a wine high in alcohol might be and remain quite sound although the volatile acid was as high as 0.15% whilst another poor in alcohol and otherwise deficient might have less than 0.10% and be quite unmerchantable. A trained palate is of the first importance here.

**Sugar.**—The sugar-content of dry wines is of the order of 0.1%. In the Paris municipal laboratory it is usual to add together the sugar and twice the alcohol, both expressed as grams per 100 c.c., and if this sum exceeds 32.5, to decide that the wine has received an addition of alcohol or sugar.

**Potassium Sulphate.**—The juice of the grape contains sulphates equivalent to perhaps 0.05% of potassium sulphate and a further amount results from the sulphuring of casks, so that wines on the average contain about 0.1%. An amount in excess of 0.2% is held to be evidence of plastering—that is, of the addition of gypsum to the must—a practice which is most common in the sherry district, though not confined to it. It is impossible to discuss here the complex reasons for the practice or the arguments which have been brought against it by hygienists. Red wines, except sweet dessert wines, must not, in Germany, contain more than 0.2% of potassium sulphate, and similar regulations apply to the sale of wines in France and Switzerland.

**Sulphurous Acid.**—In France and Switzerland, sulphurous acid in excess of 200 mg. per 1000 c.c. is forbidden, whilst the “free” sulphurous acid must not exceed 30 mg. per 1000 c.c. in France or 20 mg. per 1000 c.c. in Switzerland.

Most of the work in connection with wine has been carried out in Germany and France, and the standards suggested by French and German chemists are strictly applicable only to the wines typical of those countries, the red and white Bordeaux wines, and hocks. All these are or should be natural wines. The same standards may, with a considerable amount of caution, be applied to the wine of any other country, if that wine purports to be a natural wine. But wines are seldom so labelled; they have a distinctive name. The word “claret” has a perfectly definite significance in England. The purchaser expects a wine grown in the Bordeaux district, treated in the manner usual in that district and having the character common to wines of that name. The analyst would not pass as genuine claret a sample which showed signs of being fortified or heavily

plastered. Suppose, however, the sample is sold as "Spanish claret," and proves to be fortified and heavily plastered. The typical wine of Spain imported to this country is sherry, which is always fortified and always plastered, and it might be argued with some truth that fortifying and plastering, though they found their highest development in the manufacture of sherry, were not restricted to sweet wines but were more or less typical of Spanish practice. Certainly wines of every degree of sweetness and alcoholic strength from a typical claret up to something indistinguishable from sweet port are sold in Spain itself under one name. Such a note of warning is necessary, as wines of every type are now being produced in four continents, and it is doubtful if the purchaser of Australian burgundy or Californian sauterne has any right to expect more than a reasonable resemblance to previous consignments bearing the same label.

Port, sherry and other Spanish wines less frequently imported to this country, marsala and many Italian wines, and madeira always receive an addition of alcohol to arrest fermentation, and cane sugar is a normal addition to sparkling wines. No champagne maker would risk his valuable crop by using anything but refined cane sugar, but in Germany it has been found necessary to institute penalties to prevent the use of commercial glucose.

## CIDER

The literature of cider continues to grow, French and American chemists being responsible for most of the work, but no special analytical methods of importance have been described. Most of the methods applied to wines may be extended to cider. It is usual to calculate the non-volatile acid as malic acid and to return it as such. The statement which appears in the text-books, that the solid matter of cider differs from that of wine in the presence of malic acid, seems to rest on this convention rather than on the results of analyses directed to the differentiation of malic and tartaric acids. A method for the differentiation of malic, tartaric and succinic acids in wines, etc., has been worked out by Schmitt and Hiepe (*Zeitsch. anal. Chem.*, 1882, **21**, 534) and may be found described in Windisch (*loc. cit.*, 185). The method is said to be accurate, but it is very tedious and of doubtful utility. So far as it

has been applied to wines, the results seem to indicate that malic acid may be the chief constituent of the non-volatile acid of grape wines as well as of cider.

In the Paris municipal laboratory it is held that dry cider—that is, cider containing less than 1% sugar—should not contain less than 3% of alcohol by volume. In judging sweet cider, the sugar percentage less 1 is divided by 2 and then by 0.79, and the number so obtained added to the actual alcohol percentage. The same authority fixes the minimum extract percentage of genuine cider at 1.8, and the minimum ash at 0.17%. This latter figure should be 0.15 or less for English cider, whilst a higher alcohol percentage, say 4, might not unreasonably be insisted on. Information on the manufacture of English cider may be found in a pamphlet by F. J. Lloyd, published by the Board of Agriculture in 1903.

## POTABLE SPIRITS

The estimation of alcohol in potable spirits does not call for special consideration here. It is usually estimated from the sp. gr. of the distillate on the assumption, not quite correct, that the distillate consists solely of water and alcohol. The error introduced by this assumption is, with most spirits, very small.

**Higher Alcohols.**—The only methods which can be recommended are the Allen-Marquardt method and the British Government Laboratory method. Two others must, however, be described in some detail, partly because they have official sanction in certain foreign countries, but more particularly because the analyst may be asked by his clients to apply these specific tests, in order that the results may be compared with older records or with the numbers returned by a continental chemist to whom the same sample has been submitted.

**Allen-Marquardt Method.**—The following description differs only in detail from the official A. O. A. C. method, and differs slightly from that given by Allen in the 3rd edition of this work, in that certain suggestions of Schidrowitz (*J. Soc. Chem. Ind.*, 1902, 21, 815) have been adopted. To 200 c.c. of the sample about 1 c.c. of strong potash solution is added and the whole boiled for an hour under a reflux condenser. The liquid is then transferred to a distilling flask through the cork of which there passes, to within a few millimeters of

the bottom of the flask, a tube for the introduction of steam. Before connecting up with the supply of steam, distillation is commenced by the use of an ordinary gas-burner and continued until only about 20 c.c. are left. Steam is then turned on and the flame under the flask so regulated that the contents of the same are reduced to about 10 c.c. by the time 300 c.c. in all have passed over. The distillate is divided into two equal parts, and each is treated in the following manner, thus giving a duplicate determination of the higher alcohols:

A saturated solution of common salt is added to the liquid until the resulting mixture has a sp. gr. of at least 1.1, when it is extracted in a separator four times with carbon tetrachloride, using 40 c.c. of the tetrachloride for the first extraction; 30 c.c. for the second, 20 c.c. for the third, and 10 c.c. for the last extraction. The carbon tetrachloride now contains all the higher alcohols, and some ethyl alcohol. To remove the latter, the carbon tetrachloride is shaken with 50 c.c. of brine, and after this has been separated it is shaken with 50 c.c. of a saturated solution of sodium sulphate to remove the chloride. The carbon tetrachloride is next treated with an oxidising mixture consisting of 5 grm. of potassium dichromate, 2 grm. of strong sulphuric acid, and 10 c.c. of water. The oxidation is carried out in a flask which is connected to a reflux condenser, the liquid being kept gently boiling by means of a water-bath for at least 8 hours. Any higher alcohols extracted by the carbon tetrachloride will by this treatment be converted into their corresponding acids. After oxidation, the liquid is diluted with 30 c.c. of water, and distilled over a naked flame until only 20 c.c. remain in the flask, which is provided with a tube for the introduction of steam as in the first distillation. Steam is now turned on, and the flame under the flask so regulated that not much more than 5 c.c. remains when the total distillate measures 300 c.c. Distillation is then stopped and the distillate titrated with N/10 barium hydroxide, using methyl-orange as the indicator, and shaking the liquid thoroughly after each addition. The amount of alkali required to neutralise the liquid at this stage should not exceed 2 c.c., and generally less is required. Phenolphthalein is next added to the liquid, and the titration continued until the neutral point is reached with this last indicator. Each c.c. of N/10 alkali required in the second stage of the titration corresponds to 0.0088 grm. of higher alcohols expressed as amyl

alcohol. The alkali added when titrating with methyl-orange was formerly supposed to represent mineral acid which distilled, and is still usually not taken into account.

*Notes on the above Method.*—The brine is best made by saturating water with clean table salt, adding dilute sulphuric acid until the liquid has a distinctly acid reaction, and filtering the solution.

The carbon tetrachloride intended for use in the process must be previously purified by treatment with chromic acid mixture and subsequent distillation over barium carbonate. The carbon tetrachloride recovered at the end of the process may, after similar treatment, be used again.

The corks used in distilling the spirit must be kept separate from those used during and after the oxidation process. They are liable to absorb amyl alcohol and valeric acid, to prevent which they must all be carefully covered with tinfoil. Rubber bungs should not be used. Schidrowitz and Kaye (*Analyst*, 1905, 30, 191) recommend a condenser tube ground to fit the neck of the flask used during the 8 hours' digestion. They also recommend the use of a Young's "rod-and-disc" apparatus (*Trans. Chem. Soc.*, 1899, 75, 689) inside the 24-in. condenser-tube.

The steam used for the final distillation must be free from carbon dioxide, since phenolphthalein is to be used as indicator. This condition is easily satisfied by having the steam can or flask briskly boiling some minutes before steam is wanted.

The methyl-orange acidity was formerly attributed to hydrochloric acid, and consequently not taken into account in calculating the higher alcohols, of which the total acidity, less the methyl-orange acidity, was held to be the measure. It has been pointed out by Schidrowitz and Kaye (*Analyst*, 1906, 31, 183) that in the neutralised liquid resulting from the final titration of a carefully conducted determination, only a trace of chlorine can be found, whereas the methyl-orange acidity is almost invariably about 10% of the total acidity and is, in fact, due to the fatty acids which are not absolutely neutral to methyl-orange. They recommend calculation of the total acidity to amyl alcohol, with the reservation that, if the methyl-orange acidity much exceeds 10% of the whole, a gravimetric estimation of chlorine is indicated. The preferable plan would be to add to the number of c.c. of barium hydroxide, required in the second (phenolphthalein) stage of the titration, one-ninth or the actual

volume required in the first (methyl-orange) stage, whichever is the less, and to repeat the estimation if the methyl-orange acidity much exceeds 10% of the whole. These suggestions are placed in a note and not in the text, as evidence is lacking that they find general adoption. The use of both indicators is advisable in any case, as a check is thus provided on the manner in which the analysis has been carried out.

If an unexpectedly high value for higher alcohols is found and the methyl-orange acidity is normal, there is always a suspicion that some of the ethyl alcohol has remained in the carbon tetrachloride extract and been oxidised to acetic acid. Schidrowitz and Kaye (*Analyst*, 1905, 30, 193) say that though some ethyl alcohol is certainly extracted and is not entirely washed out, yet this, in their experience, yields but little acetic acid and is mainly converted into some non-acidic compound. It is, however, easy to determine the mean equivalent of the acids combined with barium hydroxide. To this end, the neutralised aqueous extract is separated from the carbon tetrachloride, evaporated to dryness, dried at 130° and weighed. Let the weight be  $a$  mg. and  $b$  the number of c.c. of barium hydroxide consumed in determining the total (methyl-orange and phenolphthalein) acidity, supposing the methyl-orange acidity normal.

Then the mean equivalent of the acids is given by  $10 \frac{a}{b} - 67.7$ . The equivalent thus determined will, as a rule, indicate that if acetic acid is present, its quantity must be very small. Where the methyl-orange acidity is abnormally high, the abnormal part of it may be calculated to barium chloride first, but in such a case a repetition of the whole process is indicated.

Crampton and Tolman (*J. Amer. Chem. Soc.*, 1908, 30, 98) recommend the use of an oxidising mixture consisting of 5 grm. of potassium dichromate and 5 c.c. of sulphuric acid made up to 50 c.c. with water. This quantity is used by them to oxidise the higher alcohols extracted from 50 c.c. of whisky.

**British Government Laboratory Method.**—The sample must first be freed from colouring matters and extract, for which purpose 120 — 150 c.c., or more, are diluted to nearly 200 c.c. with distilled water and gently distilled as far as possible without charring the residue. The distillate is made up to 200 c.c. with water and its alcoholic strength determined; this should be about 50% by volume and the

amount of sample distilled should be such as to give this strength. 10 c.c. of the distillate are then used for the determination of higher alcohols as described below:

*Preparation of Standard Mixture.*—1 grm. of a mixture of propyl alcohol 1 part, isobutyl alcohol 2 parts, amyl alcohol 3 parts, and capryl alcohol 1 part, is dissolved in 100 c.c. of pure 50% alcohol, this solution being further diluted to contain 0.1 grm. of the mixture of higher alcohols per 100 c.c.

*Estimation.*—10 c.c. of the distillate from the sample are treated in a flask of about 75 c.c. capacity, with 0.5 c.c. of a 1% solution of furfural and then with 10 c.c. of concentrated sulphuric acid, the latter being slowly run in to form a layer at the bottom of the flask. 10 c.c. portions of 50% alcohol containing from 0.5 c.c. upwards of the standard 0.1% solution of higher alcohols are treated in the same way. Each flask is placed in an ice-cold water-bath and shaken gently for 30 seconds, and then allowed to stand for about an hour at the ordinary temperature. The production of a reddish-violet colour indicates that the sample contains an appreciable quantity of higher alcohols, the amount of which can be estimated by comparison with the standards. The colours obtained with commercial spirits are usually very similar in tint to those obtained with the standards, though it may occasionally be necessary to remove the esters and aldehydes before making the test. The results are calculated to milligrams of absolute alcohol per 100 c.c. of absolute alcohol in the sample.

C. Simmons (*Alcohol, its Production, Properties, and Applications*, p. 422) states that the above method gives results which agree fairly well with those obtained by the Allen-Marquardt method and that it is much more expeditious. The following figures indicate the concordance obtainable by the two methods.

#### HIGHER ALCOHOLS AS PARTS PER 100,000 OF ABSOLUTE ALCOHOL

ALLEN-MARQUARDT	GOVERNMENT LABORATORY
250.9	228.0
221.3	245.6
310.6	350.8
313.1	266.6
260.0	317.5
291.4	317.5

**Röse-Herzfeld Method.**—This method, as slightly modified by Stutzer and K. Windisch, has official sanction in Germany. It de-

depends on the increase in volume of chloroform when shaken up with the spirit under certain rigidly defined conditions. For measuring the increase in volume a special apparatus is supplied by dealers, in whose catalogues it may be found figured and described as a "fusel-oil tube." From a 20 c.c. bulb springs a narrow tube, graduated throughout its length, and this tube is surmounted by a much larger bulb which is provided with a stopper. Several modifications of the tube, differing in the range and fineness of the graduations, are obtainable, but to be of any service they should show 0.02 c.c., and be readable to half this, as the total effective reading may be no more than 0.05 c.c. Alcohol, absolutely free from fusel oil, is required for control experiments; it should be at least twice fractionated over potassium hydroxide and only the middle fractions taken. For use in the test this control alcohol, as well as the spirit under examination, must be freed from carbon dioxide by boiling under a reflux condenser and diluted with great exactness to 30% alcohol by volume; that is to say the sp. gr. must lie between 0.96555 and 0.96560. The apparatus is next charged with 20 c.c. of a mixture of fuming and ordinary concentrated sulphuric acid, rotated so that the whole of the inner surface is wetted by the acid, gradually warmed up and finally kept for an hour in a water-bath not much short of boiling. It is then rinsed with distilled water and dried by a current of dry air. The apparatus is then suspended in a vessel of water at exactly 15°, and anhydrous redistilled chloroform (20 c.c.) poured in down a thistle funnel which extends nearly to the bottom of the apparatus; the object is to fill with chloroform the lower bulb, and the stem up to the lowest graduation mark, or a little above it, without wetting the upper part of the tube. After leaving the chloroform a sufficient length of time to insure its being at exactly 15°, its level is exactly adjusted by withdrawing a fraction of a drop of chloroform by means of a long capillary tube. 100 c.c. of the exactly 30% control alcohol, exactly at 15°, is then introduced, and 1 c.c. of sulphuric acid of sp. gr. 1.268. The apparatus is stoppered, turned upside down so that the contents mix in the large bulb, and shaken vigorously 150 times under water, the temperature of which must remain 15°. The apparatus is then lifted out of the water and gently inclined so that the chloroform slowly trickles back into the lower bulb; in this way a sharper line of separation is obtained. The apparatus is again suspended in a cylinder of water at 15° and after an hour (not sooner)

the reading,  $a$ , taken where the two layers meet. The whole process is next repeated with the spirit under examination. Let the reading this time be  $b$ . The German Public Health Department multiplies the number  $b-a$  by 2.22, and returns the result as parts of fusel oil per 100 c.c. of absolute alcohol in the sample. The method gives results limited in accuracy only by the manner of graduation of the instrument, when applied with every precaution to solutions in pure spirit of the higher alcohols which may occur in fusel oil. Each of the alcohols has the same or nearly the same effect on the chloroform, but commercial spirits are not simply alcoholic solutions of higher alcohols, and the actual reading is the algebraic sum of the readings which would be given by each constituent of the spirit singly. Some of these constituents may cause a contraction of the chloroform column, and Schidrowitz (*J. Soc. Chem. Ind.*, 1902, **21**, 815) has stated that certain samples of whisky actually gave negative results in his hands. The method is described at length in all German textbooks, *e. g.*, in Maercker's "*Spiritusfabrication*" (ed. Delbruck, 1903). In the papers of Schidrowitz (*loc. cit.*) and Veley (*J. Soc. Chem. Ind.*, 1906, **25**, 398) those interested will find a fairly complete set of references, but little encouragement to make use of them.

**Sulphuric Acid Method.**—This method has official sanction in France. Since some brandy shippers allege that it more often confirms their palate judgment than does the Allen-Marquardt test, it will be described here in the first place substantially as its advocates describe it (*cf.* Girard et Cuniasse, *L'Analyse des Alcools*, 1899). The criticisms to which it has been subjected cannot be incorporated in this description, since they are not helpful, but of such a nature as to absolutely discredit the method.

50 c.c. of the spirit, which by previous dilution or concentration has been brought to 50% strength, are first boiled under a reflux condenser with some reagent which will fix the aldehydes. 1 gm. of metadiaminobenzene, or 1 c.c. of syrupy phosphoric acid and 1 c.c. of aniline (Mohler, *Ann. Chim. Phys.*, 1891, **23**, 129), is generally used, but Schidrowitz and Kaye prefer calcium phenylhydrazine sulphonate (Hewitt's reagent), and this, if obtainable, is no doubt excellent for the purpose. A few pieces of pumice are added and the whole boiled for an hour. After cooling, the condenser is rearranged for distillation, and the spirit distilled until 45 c.c. have come over. The dis-

tillate is made up to 50 c.c. with distilled water and 10 c.c. transferred to a small dry flask. 10 c.c. of the purest sulphuric acid obtainable are now delivered by a pipette in such a manner that the acid flows down the side of the flask and reaches the bottom without much mixing with the spirit. The contents of the flask are then well shaken and left for an hour on the water-bath. Some workers heat the flask over a naked flame to incipient boiling and then allow it to cool, others substitute a brine-bath for the water-bath. After cooling, the colouration developed is compared with that given under the same conditions by a standard solution of isobutyl alcohol in pure 50% ethyl alcohol. This solution is made by dissolving exactly 0.5 grm. of pure isobutyl alcohol in 1000 c.c. of 50% ethyl alcohol. For the purpose of strict comparison 50 c.c. of this solution should be distilled with the chosen de-aldehyding reagent and the first 45 c.c. of the distillate collected and diluted to 50 c.c. 10 c.c. of this solution are treated with 10 c.c. of sulphuric acid and heated exactly like the spirit under examination. The resulting liquid constitutes the colour standard. If the tints of the standard and of the assay liquid are identical, the 50% spirit under examination may be returned as containing 0.083% of higher alcohols, since average fusel oil is said to develop only 0.6 of the colour given by isobutyl alcohol. Results are more conveniently returned in milligrams per 100 c.c. of absolute alcohol. Thus a sample exactly matching the standard would be returned as containing 167 mg. per 100 c.c. of absolute alcohol. If, as is usual, the tints of the standard and assay liquid differ, it is necessary to compare them accurately. Numerous special colorimeters have been devised with the object of facilitating this comparison but it is to be noted that the colour does not vary directly with the content of higher alcohols, and that something more than a rule-of three sum is required in calculating the results. Suppose, for example, that a layer of the assay liquid has the same intensity of colour as a layer of the standard only half its depth, the number to be returned is not 83, but 116 mg. of higher alcohols per 100 c.c. of absolute alcohol. Girard and Cuniasse (*loc. cit.*) give a curve and table connecting "apparent" and "real" content of isobutyl alcohol, but as this is only a secondary constituent of fusel oil, the following table is perhaps more useful.

RATIO OF INTENSITY OF COLOUR OF ASSAY LIQUID TO THAT OF STANDARD	Mg. OF HIGHER ALCOHOLS PER 100 C.C. OF ABSOLUTE ALCOHOL IN SAMPLE
0.1	53
0.2	77
0.3	92
0.4	105
0.5	116
0.6	127
0.8	147
1.0	167
1.2	184
1.4	201
1.6	218
1.8	235
2.0	252

The above table is calculated from the curve of Girard and Cuniasse on the assumption that "average" fusel oil produces only 0.6 as much colour as its own weight of isobutyl alcohol under the conditions of the test. The last figure of each of the numbers in the right-hand column has no justification, but the writer has not yet met with any chemist who is content with a 0 in this place. "Average" fusel oil is but a figment of the imagination. The proportions of the higher alcohols present differ with the raw material and manner of distillation of the spirit, and each one has its own capacity, greater or less, for producing colour in this test. Experiment shows that the numbers obtained in this test bear no constant relation to those obtained by the Allen-Marquardt method, which, whatever its imperfections, is based on scientific principles. The most serious criticism to which the test has yet been subjected, however, is that of Veley (*J. Soc. Chem. Ind.*, 1906, 25, 400), who found that isobutyl alcohol itself, if carefully purified, gives no coloration with pure sulphuric acid. The reason for devoting so much space to so unsatisfactory a test has been already given, and is sufficient. Even Veley says the test is capable of giving valuable information. No one will refuse a chemist the right to make any test which aids him in forming a judgment regarding a sample, but if the number obtained by the sulphuric-acid test is returned in a certificate as a measure of the higher alcohols, it is reasonable to require the addition of the words "colorimetric method," since the colour may be and probably is the measure of something else and not at all of the higher alcohols.

It has already been said that the Allen-Marquardt method is the only one which can be generally recommended. No method for the

estimation of such a variety of substances as is included under the heading of "fusel oil" can be entirely satisfactory, and isopropyl alcohol is theoretically not estimated by the Allen-Marquardt method, since on oxidation it yields acetone and no acid. Jenks and Bedford (*J. Soc. Chem. Ind.*, 1907, **26**, 123) find that the Allen-Marquardt method greatly underestimates every constituent of fusel oil except amyl alcohol, and they have devised a method which they allege enables them to differentiate between amyl alcohols on the one hand and butyl and propyl alcohols on the other, but little experience has yet been gained with the method and, so far as the writer knows, it is used only by its authors.

**Acids and Esters.**—100 c.c. of the spirit are distilled until only about 10 c.c. remain; distillation is then continued by passing in steam, free from carbon dioxide, as in the Allen-Marquardt process for the estimation of higher alcohols. The bulk of the distillate should be about 150 c.c. and the residue left in the flask not much more than 5 c.c. This residue may be diluted with water, the fixed acid determined by titration with  $N/10$  alkali, using phenolphthalein as indicator, and the result calculated in terms of tartaric acid. Only spirits which have been stored long in wood contain any appreciable amount of fixed acid.

The distillate contained in a Jena flask is exactly neutralised with sodium hydroxide, phenolphthalein being used as indicator, and the volatile acid calculated as acetic acid, though higher acids are certainly present in some spirits. A further 10 c.c. of  $N/10$  alkali is now added and the whole boiled under a reflux condenser for half an hour. After cooling, 10 c.c. of  $N/10$  acid are added and then  $N/10$  alkali to exact neutralisation. The amount of  $N/10$  alkali required in this last titration is calculated in terms of ethyl acetate, though more complex esters are, no doubt, generally present.

Allen preferred to remove aldehydes before proceeding to the estimation of esters. Though this is desirable on theoretical grounds, in practice the error involved by neglecting the action of the aldehydes on the standard alkali is very small, whereas the use of any of the de-aldehyding reagents suggested may introduce errors of unknown magnitude. Hewitt's reagent (sodium or calcium phenylhydrazine-*p*-sulphonate) is the least objectionable of these reagents, but Hewitt himself (*Analyst*, 1905, **30**, 153) does not recommend its use in this connection.

**Furfural.**—In a colourless spirit this is easily estimated by comparing the tint produced in the liquid by the addition of aniline acetate with that produced in a standard solution of furfural in pure 50% alcohol. The alcohol used for preparing and diluting the control solution must be free from aldehyde. It is digested with potassium hydroxide and fractionated, and only the portion boiling between 78° and 80° collected. If this gives any colouration with aniline acetate, the treatment should be repeated or recourse may be had to any of the de-aldehyding reagents already mentioned. In the reviser's experience the glacial acetic acid supplied to analytical chemists never contains furfural or even traces of this substance, present in commercial acid, which develop a yellow colour with aniline. It is convenient to boil for a few minutes equal volumes of aniline, acetic acid and water. The mixture, when cool, constitutes the reagent, and the boiling effectually destroys any furfural which might be present in the acid. The most convenient strength for the control liquid is 0.05 grm. furfural per 1000 c.c. of 50% alcohol, and it is of course made by diluting a stronger solution. Since the colouration is in some measure dependent on the alcoholic strength of the liquid, it is advisable, when the spirit under examination differs much from 50% strength, to dilute the control liquid with water or pure alcohol until its alcoholic content approximates that of the sample. To 20 c.c. of the spirit and 20 c.c. of the control solution, each contained in Nessler glasses, 1 c.c. of the aniline acetate solution is added, and after 10 minutes the tints compared. Some of the darker solution is now withdrawn until, on looking down the tubes, the tints appear identical. If the control liquid was diluted to adjust its alcoholic content, this must not be overlooked in the calculation, which is otherwise similar to that applied in nesslerising.

When, as is usual, the spirit has considerable colour, this must in some manner be removed. Hewitt (*J. Soc. Chem. Ind.*, 1902, 21, 98) recommends distilling nearly to the last drop, adding pure dilute alcohol to the distilling flask, and again distilling nearly to the last drop, and so on three or four times. The united distillates are then made up to some definite volume. Schidrowitz (*J. Soc. Chem. Ind.*, 1902, 21, 816) strongly criticises this procedure, mainly on the ground that furfural may be formed during the distillation. He prefers to decolourise as far as possible with lead acetate, and then to

add the aniline reagent to the liquid under examination and to the control. If the shades (not the intensity of colour) differ, dilute tincture of galls is added to the control until they match. The tincture of galls is added after, and not before, the reagent because it is intended to neutralise not only the tint remaining in the spirit after treatment with lead acetate, but also the yellow colour which certain aldehydic substances give with aniline. To 20 c.c. of the sample, Schidrowitz adds a few drops of basic lead acetate solution, shakes, adds enough saturated potassium sulphate solution to precipitate the excess of lead, filters and proceeds as above described.

**Aldehydes Other than Furfural.**--Many methods for the estimation of aldehydes in potable spirits have been described. The only one in common use, however, is a colourimetric estimation by means of Schiff's reagent. The usual formula for the reagent is:

- 0.15 grm. of fuchsin in 150 c.c. of water,
- 100 c.c. of sodium hydrogen sulphite solution (sp. gr. 1.36),
- 10 c.c. of concentrated sulphuric acid.

As the presence of much mineral acid greatly reduces the sensibility of the reagent, the following modification is recommended:

0.2 grm. rosaniline base is dissolved in 20 c.c. of a cold saturated solution of sulphurous acid; if the colour is not discharged after 24 hours, a further 10 c.c. of sulphurous acid is added; after a further 24 hours the colour will usually be discharged, but if not, more sulphurous acid is added and the solution when finally decolourised is diluted to 200 c.c. with water. Some samples of rosaniline yield yellowish-brown solutions which cannot be entirely bleached, but when diluted to 200 c.c., the colour, even of bad samples, is seldom of serious account.

A control solution of acetaldehyde in pure 50% alcohol is required. A convenient strength is 0.2 grm. acetaldehyde per 1000 c.c. The alcohol must be freed from aldehyde similarly to that used in preparing the furfural control. It is convenient to prepare a stock which reacts neither with Schiff's reagent nor with aniline. The official A. O. A. C. method of obtaining aldehyde-free alcohol is as follows: After distilling the alcohol over sodium hydroxide, 3 grm. of metaphenylenediamine are added and the mixture allowed to stand for several days at room temperature or boiled under a reflux condenser for several hours, and then distilled slowly, the first 100 c.c. and the last 200 c.c. being rejected.

The official A. O. A. C. method for preparing a standard aldehyde solution is as follows: Grind aldehyde ammonia in a mortar with ether and decant the ether, repeating this operation several times; then dry the purified material, first in a current of air and then in a vacuum over sulphuric acid. Dissolve 1.386 gm. of this substance in 50 c.c. of 95% alcohol, purified from aldehyde; to this solution add 22.7 c.c. of N/1 sulphuric acid, made with alcohol instead of water, make up to 100 c.c. and add 0.8 c.c. to compensate for the volume of the ammonium sulphate precipitate. Let the liquid stand overnight and then filter. The solution contains 1 gm. of aldehyde in 100 c.c. and keeps well.

The most convenient standard is prepared by adding 2 c.c. of the above solution to 100 c.c. of 50% (by volume) alcohol; 1 c.c. of this dilute solution contains 0.0002 gm. aldehyde. This dilute solution does not keep.

The test is carried out by adding to 20 c.c. of the liquid under examination and to 20 c.c. of the control solution, 5 c.c. of the reagent, and comparing the tints produced after 20 minutes. Portions of the darker are withdrawn until, on looking down the tubes, the tints appear equal. Dubosc's or other colourimeter is used by those who make many of these determinations, but the chemist in general practice may use Nessler glasses, and calculate on the assumption, not quite true, that the intensity of colour is proportional to the amount of aldehyde present. The influence of furfural may be neglected, since it gives a very faint colouration with Schiff's reagent, compared with that given by acetaldehyde. It is desirable that the solution under examination and the control solution should be of approximately the same alcoholic strength, and this is effected by adding to one or other of them water or pure alcohol.

Highly coloured spirits are best treated by Schidrowitz's method, described under Furfural. The spirit is decolourised as far as possible with basic lead acetate, the excess of the latter removed by the addition of potassium sulphate, and the liquid filtered. The control is then coloured with tincture of galls until it exactly matches the sample, Schiff's reagent added to both control and assay liquid and the comparison made after 20 minutes.

**Non-volatile Residue.**—This is sometimes of importance. When freshly distilled, spirits contain no trace of non-volatile matter. When kept in casks they take up more or less fixed matter, but the

amount rarely exceeds 100 gr. per gallon. The fixed matter may include, among other substances, tannin, colouring matter, sulphates and traces of sugar. The proportion of non-volatile matter in spirits is ascertained by evaporating 50 or 100 c. c. to dryness on a water-bath. Some indication of its nature may be obtained by tasting the residue. On ignition in the air, any zinc, lead, or copper present in the spirit will be left as an oxide. Very sensible traces of these metals may be present accidentally, and there is good evidence that their salts were in the past occasionally used as adulterants. Occasionally, clarifying materials containing lead acetate have been employed. Alum was also used occasionally. The reaction of the ignited residue should be observed, as, if alkaline, an alkaline carbonate, acetate, tartrate, etc., must have been present.

**Sulphates** will be detected on adding barium chloride to the diluted spirit. Free sulphuric acid has been met with in whisky, and is said to have been used formerly for adulterating gin. This is extremely improbable. The presence of free sulphuric acid may be detected by the methods used for examining vinegar for mineral acids.

**Tannin** is often present in brandy, being chiefly extracted from the casks used for storing. Sometimes it is purposely added in the form of tincture of galls or oak-bark. It may be detected by the darkening produced on adding ferric chloride to the spirit, and any reaction thus obtained may be confirmed by boiling off the alcohol from another portion of the spirit and adding solution of gelatin to the residual liquid, when a precipitate will be produced if tannin be present.

A few analyses of spirits are given here, not to serve as "types" nor to prove the folly of referring spirits to types, but to give some idea of the results to be expected. Girard and Cuniasse, at the end of their book and elsewhere, have published a large number of spirit analyses; the most interesting of their numbers are those which relate to brandy, but they give several examples of French industrial alcohol. Probably the whiskies selected by Schidrowitz (*J. Soc. Chem. Ind.*, 1902, 21, 818) are more typical of the spirit consumed in Great Britain than are the whiskies on which continental chemists report from time to time. Vasey (*Analysis of Potable Spirits*, London, 1904) gives a number of analyses, some from continental sources but many original, whilst in König's "*Chemie der menschlichen Nahrungs- und Genussmittel*" there are many more. Reference to

Vasey may lead the analyst to suppose that the judgment of spirits is comparatively simple, and Girard and Cuniasse appear to base confident judgments on analytical data, but some of their own selected analyses invalidate the standards they suggest. Both Vasey and Girard and Cuniasse, it is true, assume that the analyst will call on his palate to aid him in his judgment, but they may fairly be quoted as representing the school which believes most strongly in the ability of the chemist, *qua* chemist, to decide whether spirits are "genuine" or otherwise. As a corrective to too great confidence in numbers, the analyst may be referred to a communication by Windisch (*Zeitsch. Unters. Nahrungs- und Genussm.*, 1904, 8, 465) and to a paper on "Brandy," by Hehner (*Analyst*, 1905, 30, 36).

## RESULTS OF ANALYSES OF POTABLE SPIRITS

	Alcohol per cent. by vol.	Milligrams per 100 c.c. of absolute alcohol				
		Higher alcohols	Esters	Acid	Furfural	Aldehydes excl. furfural
1. Brandy, genuine grape, 2 years	64.4	253	136	77	1.3	19
2. Brandy, genuine grape, 16 years	61.1	95	81	59	1.0	24
3. Brandy, genuine grape, 35 years	47.5	345	133	202	1.2	48
4. Brandy, admittedly blended with patent spirit	50.0	60	67	58	0.9	18
5. "Brandy," admittedly flavoured patent spirit (no grape)	50.0	18	32	19	0.2	6
6. Sold as "cognac"	64.0	Nil	14	11	0.3	2
7. Whisky, malt, new	62.8	189	70	16	4.4	11
8. Whisky, malt, 4 years	60.5	217	95	55	3.0	23
9. Whisky, grain, new	61.5	76	48	Nil	Nil	5
10. Whisky, grain, 4 years	59.7	77	77	11	Nil	11
11. Rum, Jamaica, genuine	69.5	94	440	176	2.9	22
12. Rum, admittedly blended with patent spirit	36.0	114	83	127	0.0	11
13. Sold as "rum"	55.0	8	45	65	0.6	6
14. Gin		45	37	Nil	Nil	2
15. Highly rectified spirit		3	3	3	Nil	0.1

**Brandy** is usually defined as a spirituous liquid, distilled from wine and matured by age. The best, that is the most palatable and valuable brandies, are no doubt produced in this way, but it is difficult, if not impossible, for a chemist to decide with certainty whether a particular sample of "brandy" is properly so described. It is true that on the average brandies contain 80 to 100 mg. of esters per 100 c.c. of absolute alcohol, and that some, including some of the

finest, contain much more, but some genuine wine brandies contain less than half this amount, and there is nothing to prevent a distiller from obtaining pure alcohol from wine except the consideration that pure alcohol is flavourless and not saleable at the price obtained for less pure distillates. On the other hand, except as regards the all-important flavour, there is no difficulty in producing a spirit, innocent of grapes, but complying with any of the standards which have been laid down. A little rum, with 400 mg. esters per 100 c.c., will supply the necessary esters to a large bulk of silent spirit, and so on with other constituents. The sum of the higher alcohols, esters, acid, aldehydes and furfural is usually over 300 mg. per 100 c.c. of absolute alcohol, but it is generally agreed now that no rules can be laid down for this total which was formerly spoken of as the "coefficient of impurities."

Nor is it easy to decide on the age of brandy or other spirit. Some oxidation with formation of aldehyde and acid is to be expected, and the increased acid determines some further esterification, but as spirits start with such widely different compositions, it is possible for one 20-year-old brandy to be indistinguishable analytically from a new brandy from another still. Number 2 is a case in point. The low ester number, which would cause some to doubt the age of this sample, is no doubt due to the manner of storage which was such that comparatively little oxidation took place, with consequent small increase of the acidity on which the degree of esterification probably depends. It is highly improbable that any distiller of genuine grape brandy would deliberately refine his product so as to get an article like No. 6, but the figures given do not prove that the spirit was other than brandy as defined by the British Pharmacopœia.

**Whisky.**—Like other potable spirits whisky is more or less aqueous alcohol, containing a small proportion of other matters which give it the characteristic flavour associated with the name; flavours, perhaps would be more correct, since several types of whisky are distinguished by makers and drinkers of the beverage. It may be derived exclusively from malt and distilled in the comparatively simple pot still, or mainly from raw grain and distilled in a patent still which is capable of bringing about very complete rectification. A Royal Commission, which issued its report in 1909 (*Blue Book*, Cd. 4796), concluded that whisky is a spirit obtained by distillation from a fermented mash of cereal grains saccharified by the diastase of malt;

that Scotch whisky is whisky as above defined, distilled in Scotland; and that Irish whisky is whisky, as above defined, distilled in Ireland. It has been contended that only pot-still spirit can be termed "whisky," but the Commission did not support this view. The whisky distiller does not work his patent still so as to bring about the maximum degree of rectification of which it is capable, and in pot-still distillation about two-thirds of the total volatile impurities are eliminated with the pot ale and spent lees, so that the difference between pot-still and patent spirits is not necessarily so great as is sometimes supposed. It is incorrect to speak of the pot still as an apparatus in which little or no fractionation occurs, but equally incorrect to lay stress on the temporary separation of the distillate into three fractions as do Schidrowitz and Kaye (*J. Inst. Brew.*, 1906, **12**, 496). Of these fractions the first and last are added to the next charge to recover the alcohol contained in them, and the only certain measure of impurities eliminated is the amount contained in the pot ale and spent lees, which with whisky are the sole ultimate products of pot-still distillation. Schidrowitz and Kaye have shown that the esters of the foreshots and feints may be partially hydrolysed on repeated distillation, and that aldehyde may be oxidised to the much less volatile acid is probable, but their figures, based on a single run, scarcely justify their conclusion that only 10% of the total impurities find their way into pot-still whisky: in fact, they may equally well be made to support an estimate of 30%.

A large proportion of the whisky sold, and approved by its purchasers, is a blend of grain whisky containing notably less impurities than Nos. 9 and 10 with a pot-still whisky containing notably more impurities than Nos. 7 and 8.

AMERICAN WHISKY is the subject of a special paper (*J. Amer. Chem. Soc.*, 1908, **30**, 98) by Crampton and Tolman, who show that its composition may range within wide limits. Thirty whiskies were examined and, what is more important, were preserved in bonded warehouses in barrels, which were opened once a year for 8 years and a sample from each withdrawn and analysed. No such thorough investigation into the effects of long storage in wood has been made before, and if it is said that no new information has been brought to light, the answer is that this investigation transforms into facts what were previously no more than reasonable hypotheses. The result of this investigation is to establish the following facts:

Water passes more easily than alcohol through the pores of the wood, with the result that the alcoholic strength of spirits stored in wood increases about 1% per annum. The increase in the percentage of higher alcohols with age is entirely explained by the diffusion of water and ethyl alcohol through the pores of the wood, which appears to be practically impervious to the higher alcohols. The other "impurities" do actually increase in amount, the increase being comparatively rapid during the first 3 or 4 years and after that proceeding very slowly. The work of Crampton and Tolman also establishes the fact that the source of furfural in whisky is two-fold; it may be derived from the grain of the mash or from the charred wood of the barrel. The aroma and flavour of the whisky are derived from the charred interior of the barrel.

**Rum**, especially Jamaica rum, is usually characterised by its high content of esters and volatile acid and by its flavour.

**Gin** is made by flavouring highly rectified spirit with oil of juniper berries or other substances, with or without the addition of sugar. Previous to sale the gin is broken down considerably by addition of water.

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# YEAST

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REVISED BY EMIL SCHLICHTING

**General.**—Yeast must be considered from two points of view, as a plant and as a ferment, based upon the fact that, while the yeast cells develop and propagate, the yeast has only little or no fermentative activity.

**Physical Properties.**—According to Schönfeld, Hinrichs and Rossmann (*Wochenschrift Brau.*, 1910, **27**, 493), the four main characteristics of a “*top fermenting yeast*” are: (1) branched budding fermentation; (2) little fermentation of melitriose; (3) formation of surface yeast at room temperature; (4) milky mixture in water.

Schönfeld and Hirt (*Wochenschrift Brau.*, 1912, **29**, p. 174) point out that considerable difference exists among the various yeasts in their mode of settling or sedimentation, some separating out in heavy lumps whilst others subside as a fine, loose dust. According to these authors, lumpy yeasts contain a larger percentage of phosphoric acid and magnesia. The distinctive character of settling is also greatly influenced by the nature of the proteins contained in the nutritive medium. The more complex their molecular composition the greater the tendency of the yeast to separate in solid, lumpy formation.

**Classification.**—In the latest systematizing of yeasts. Hansen (F. Lafer, *Techn. Mykologie*, IV, p. 172) distinguishes between the following groups:

*Saccharomyces.*

*Zygosaccharomyces.*

*Saccharomycodes.*

*Saccharomycopsis.*

*Pichia.*

*Willia.*

The *Schizosaccharomyces* are not included in this family. Simultaneously with these changes in the grouping of yeasts, several of the known species have been given new names. The most important of these changes are:

Old name	New name
<i>Saccharomyces Ellipsoideus</i> I.	<i>Saccharomyces Ellipsoideus</i> .
<i>Saccharomyces Ellipsoideus</i> II.	<i>Saccharomyces Turbidans</i> .
<i>Saccharomyces Pastorianus</i> I.	<i>Saccharomyces Pastorianus</i> .
<i>Saccharomyces Pastorianus</i> II.	<i>Saccharomyces Intermedius</i> .
<i>Saccharomyces Pastorianus</i> III.	<i>Saccharomyces Validus</i> .
<i>Saccharomyces Membranefaciens</i> .	<i>Pichia Membranefaciens</i> .
Carlsberg Yeast I.	<i>Saccharomyces Carlsbergensis</i> .
Carlsberg Yeast II	<i>Saccharomyces Monacensis</i> .

**Chemical Composition.**—The characteristic odour of yeast is ascribed (Delbrück, *Brau. Lexikon*, 1910, 455) to a colourless ethereal oil, the odour of which, in concentrated form, resembles the flavour of hyacinth.

Henneberg (*Wochenschrift Brau.*, 1910, 27, 429) has found that a definite relation exists between the amount of protein and glycogen present in yeast and that when the former rises above 53%, the latter is reduced to almost nil.

Salkowski (*Chem. Centralbl.*, 1911) has determined the amount of yeast gum to be about 5%. He has further shown that the gum consists of a dextro-mannan, which on hydrolysis forms dextrose and mannose.

**Fermenting Enzyme.**—A. V. Lebedew (*Annales de l'Institut Pasteur*, 1912, 26, 8) has demonstrated that the active fermenting enzyme can be extracted from dried yeast by simple maceration with water at a temperature from 25° to 30° C. for a period of 2 hours.

The process of decomposition of sugar into alcohol and carbon dioxide is a very complicated one (Delbrück, *Brauerei Lexikon*, 1910, 865). According to Harden and Young, the transformation is carried out by the zymase proper and its co-enzyme, which is considered to be an easily saponified ester of phosphoric acid.

The destruction of the activity of pressed yeast juice is mainly ascribed to the action of a saponifying enzyme or lipase upon the co-enzyme; this is also accompanied by the action of a proteolytic enzyme, endotryptase, upon the zymase. According to Rogers and Jensen (*Centralblatt für Bakteriologie*, 1911, 29), the enzyme lipase plays an important part in the decomposition of butter by the action of certain species of *Torula*; and Delbrück (*Wochenschrift Brauerei*, 1902, 19, 25) has expressed the opinion that the gly-

cerin produced during fermentation is a product of the action of lipase upon fatty matter. Inactive yeast juice may be regenerated by the addition of yeast water, if such addition is made shortly after the inactivity has set in.

Among other enzymes, the presence of which in yeasts has lately become apparent, may be mentioned *Emulsin*. Henry and Auld (*Proc. Royal Society*, 1905, 76), found that certain yeasts were able to decompose amygdalin in the presence of toluol.

**Nutrition.**—The most important sources of carbon for the yeasts are as follows: sugars of the groups  $C_6H_{12}O_6$  and  $C_{12}H_{22}O_{11}$ , acetates, lactates, tartrates, malates, succinates, glycerols; further lecithin, asparagin, and peptones. It has also been shown by Lindner and Cziser (*Wochenschrift für Braueri*, 29, 1-6) that ethyl alcohol is an excellent source of carbon.

The sources of nitrogen may be classified into inorganic and organic compounds; in the first group are mentioned only ammonia salts, as the nitrates are only assimilable in rare cases. Among the organic nitrogen sources the most important are: albumins, albumoses, peptones, amides and amino-acids.

Oxygen is obtained by the yeasts from air, water and various organic compounds.

The breathing process of yeast goes on continually during the life of the yeast; but it is reduced to a minimum during the fermenting process.

**Fermentation.**—The fermentation process proper is the splitting up of certain carbohydrates into alcohol and carbonic acid gas.

As already pointed out by Pasteur, this process takes place more vigorously under partly anaerobic conditions.

When yeast is grown under extreme aerobic conditions, only a small amount of alcohol is formed, the yeast consuming the main part of the sugar for its own nourishment and multiplication.

The chemical formula according to which the alcoholic fermentation proceeds was originally propounded by Gay-Lussac as follows:



Later investigations, however, have shown that this process is far more complicated than indicated by this formula. Several formulæ have been suggested by various investigators; but as these

are all more or less theoretical and hypothetical, it is not considered of any practical value to embody them in this volume.

**Testing of Yeast.**—C. Nagel (*Brennerei Zeitung*, 1911) has modified the Hayduck method of estimating the fermenting power of yeast for baking purposes by changing the composition of the solution employed in the following manner:

400 c.c. of 10% cane-sugar solution to which are added: 2 grm. of potassium hydrogen phosphate; 1 grm. of ammonium hydrogen phosphate; 0.25 grm. magnesium sulphate; 0.20 grm. calcium sulphate. The valuation of the yeast is based upon the amount of carbon dioxide generated within 2 hours, and may be expressed as follows:

	FERMENTING POWER
1000 c.c. of CO <sub>2</sub>	good
800 to 1000 c.c. of CO <sub>2</sub>	medium
less than 800 c.c. of CO <sub>2</sub>	poor

**Dry Yeast.**—According to Hayduck and Bulle (*Wochenschrift Brau.*, 1912, vol. 29, 489), when in the drying process of the yeast proper the moisture content drops below 25 or 30%, all vegetative cells are killed. That sometimes such yeast will start to grow again is entirely due to some surviving spores.

In order to produce a dried yeast with about 90% of living cells, it should be mixed with 10% of cane-sugar at a temperature of 50° and the mixture should be dried at this temperature on gauze, the period of drying being about 3 hours. The drying may be accelerated by a strong air current; as the sugar added is fermented during the drying process, little or no sugar will be found in the finished product.

The drying of yeast has lately become a general practice, especially in Europe, for economic reasons and various forms of apparatus have been constructed for this purpose. It has been definitely established that yeast after being freed from its bitter substances is an admirable substitute for meat in the production of extracts, bouillon, etc. The nitrogenous substances in this "*nutrient yeast*" are almost entirely soluble and assimilable. Their nutritive value is very high, approximately three times that of fresh meat (Hayduck, *Jahrbuch Vers. und Lehranst. Brau.*, 1911, 286).

Hayduck (*Jahrb. Vers. Lehranst. Brau.*, 1913, 536) has found that yeast taken from the bottom of a fermented liquid contains an

appreciable amount of alcohol, which, under the conditions ordinarily prevailing in breweries, amounts to about 3% of the total yeast. As soon as methods for the separation of this alcohol are perfected, it will constitute an important by-product of the yeast-drying process.

Yeast is an organised ferment, belonging to a class of fungi grouped botanically as "budding fungi" and generally characterised by their faculty of causing alcoholic fermentation in a saccharine solution and by their mode of propagation by "budding," although at times propagation by fission has been observed.

The yeast fungi constitute the genus "Saccharomyces," which is again subdivided into many species. The Saccharomycetes, or yeast fungi having the distinctive faculty of forming endospores, are the only and most important ones for the fermentation industry, whilst all other yeasts and many Saccharomycetes are of no value to the industry and arts; in fact, some of these are frequently detrimental. The yeast plant is abundantly distributed throughout the vegetable kingdom and in the air.

**Physical Appearance.**—As observed in the distillery and brewery, it forms a pale yellowish-white frothy mass with a peculiar ethereal odour and generally bitter taste. The brewer distinguishes between *top* and *bottom* fermenting yeast; the former acts at temperatures of 18° to 25° and appears at the surface of the liquid whilst the latter ferments at temperatures from 4° to 10° and settles at the bottom of the fermenting liquid.

**Microscopical Structure.**—Examined under the microscope, yeast appears in the form of many cells of 7 to 10 $\mu$  in diameter.

They are seen as either single cells or colonies; their shape differs with the various species from a round to oblong, sometimes elliptical form, but even this variation of form occurs in the same species, so that, according to Hansen, a grouping or differentiation of species by this means alone becomes almost impossible. The yeast cell consists of a colourless cell wall with equally colourless cell contents; the latter consisting of

- (a) Protoplasm,
- (b) Nucleus,
- (c) Vacuoles, and
- (d) Some other granular enclosures of various description.

**The Cell Wall.**—This is very thin, generally 0.5 to 1.0 $\mu$ ; but this thickness is observed, according to Will, mostly in yeasts which have

been accustomed to ferment very concentrated worts. The cell wall is generally supposed to consist of two or more layers; it does not turn blue with a mixture of iodine and sulphuric acid, which colours the membrane a brownish-yellow as in the case of other fungi, proving that the cellulose of yeast is not closely related to starch. The several layers of the cell wall can be made visible, according to Will and Casagrandi, by a protracted treatment with 1% chromic acid or with concentrated hydrochloric acid. The outer layers of the thicker membranes are sometimes shed by the cells, as has been observed by Lindner and Will.

**Chemical Nature of Cell Wall.**—According to investigation, *true* cellulose does not occur in the cell wall of yeast as it resists the solvent action of ammoniacal cupric hydroxide (Schweitzer's reagent) and it is not coloured blue by zinc iodochloride. After treatment with 4% hydrochloric acid only, the cell wall is stained by Hanstein's aniline violet (according to Becker); this is disputed by Casagrandi.

Lindner found that as an exception, the membrane of spores of *Schizosaccharomyces octosporus* is coloured blue by a solution of iodine in potassium iodide. The yeast cell membrane will be dissolved by concentrated sulphuric and chromic acids, but not by any dilute acids. Alkalies and Schultze's macerating fluid have a tendency to clear up the cell wall.

According to Will and Casagrandi, the two layers of thick membrane differ in their behaviour towards chromic acid, the inner one being dissolved somewhat quicker than the outer; Casagrandi therefore assumes that the cell wall, as shown by its resistance towards staining agents and solvents, consists mostly of pectose or a substance closely resembling pectins.

Salkowski succeeded in isolating from yeast by means of potassium hydroxide two cellulose-resembling bodies, which upon hydrolysis produced dextrose or glucose and mannose. Hansen later observed a certain relation between the cell wall and the so-called gelatinous network, which is formed under certain conditions. He found that the membrane excreted a mucilage which on drying formed the peculiar network. This occurs in spore cultures on gypsum blocks and, according to Jörgensen, also if the yeast dries between blotting-paper. Washing removes the gelatinous network, but it is reproduced, provided the washing is not carried too far. The network is precipitated by a 5% borax solution similarly to plant gelatins;

PLATE I.

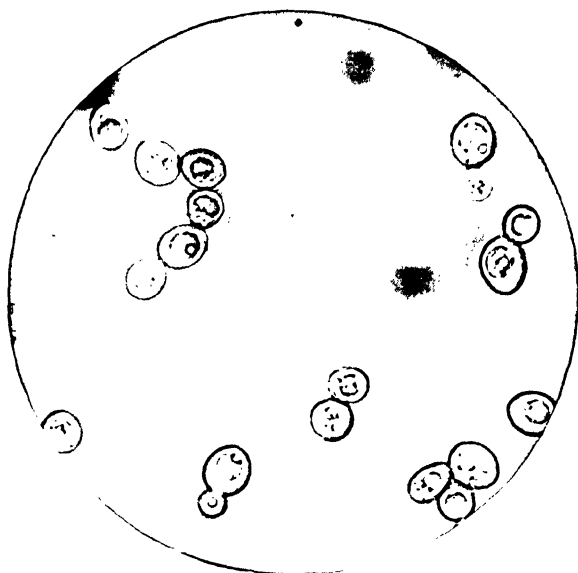


FIG. 1.—Ale Yeast. Top fermenting.

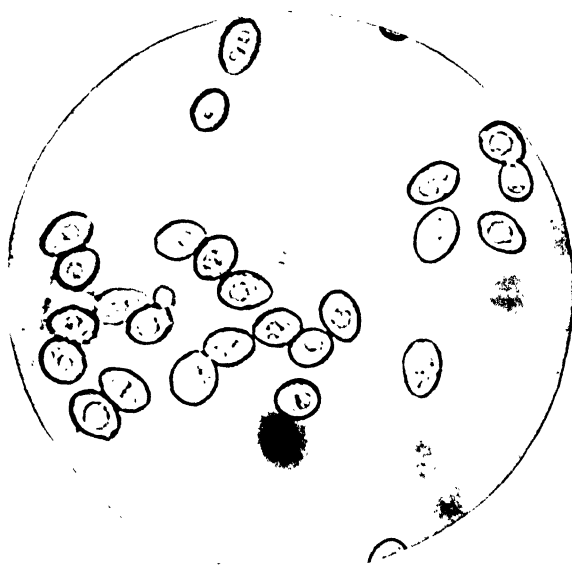


FIG. 2.—Lager Yeast. Bottom fermenting.

(To face page 272.)



this is utilised in practice for facilitating the settling and pressing of yeast by the addition of borax. Will considers the formation of this network due to a gelatinisation of the cell membrane, the protein content also taking an active part. The exact constitution and formation of this network under certain conditions and in various yeasts have not been established, but former investigators, *i. e.*, Nägeli and Pasteur, have attributed to yeast the faculty of separating and excreting protein bodies such as peptones.

**Cell Contents.**—The cell nucleus is difficult to distinguish in the living cell. It may be made visible by staining. It is generally spherical, sometimes disc-shaped. Its diameter is about one-third of the whole cell.

According to investigations of Janssens and Leblanc, there exists only one nucleus in each cell. Dangeard, Janssens and Wagner assert that it encloses a nucleolus or granular body, possessing a membrane. The intermediate space consists of a fine network of granular protoplasm. At the end of fermentation, at a stage of exhaustion, vacuoles appear filled with a fluid of unknown composition, differing from protoplasm in lower refraction. They sometimes occupy the largest portion of the cell and also contain crystalline enclosures. Frequently they have been observed to contain very small granular bodies which are in constant motion (Brownian movement). Küster considers them decomposition products of protoplasm, of a semi-liquid consistence, which eagerly absorb stains.

Strange refractive bodies are often seen in the protoplasm, appearing generally at the end of fermentation; these were formerly considered as *oil drops*; they are now called *granules*; their number and size differ considerably in the various cells; they are at times round and then angular. According to Will, their membrane consists of protein matter, with a similarly constituted interior network; their contents are of a fatty nature; as it may be removed by fat-dissolving reagents (ether, chloroform, alkalies, alcohol, petroleum spirit). The protein cell wall is dissolved by concentrated sulphuric acid, the oily drops flow together, and become first green, then bluish-green and finally black. Absolute alcohol, added to yeast, causes the cells to shrink and they are soon killed. Dead cells are generally distinguished from live ones by their greater absorbing faculty and ease of staining.

**Chemical Composition of Yeast.**—The percentage composition of yeast shows only very slight differences in the analyses of top and bottom yeasts. The nitrogen is usually somewhat higher in top yeast, but generally the composition will depend on the nutrition. According to Mitscherlich, Schlossberger, Dumas, Wagner and Liebig, the ash-free dry substance of yeast has the following composition:

	CARBON	HYDROGEN	NITROGEN
Top yeast,	48.64	6.76	11.46
Boston yeast	44.99	6.72	8.73

Older yeast, according to Schlossberger, is generally somewhat poorer in nitrogen, owing to decomposition of its cell contents.

The moisture ranges from 75 to 83%.

The sulphur ranges from 0.39 to 0.69%. (Liebig.)

**Ash of Yeast.**—Investigators differ considerably in their results regarding the ash content. This is stated for top yeast to be from 2.5 to 11.5%; for bottom yeast from 3.5 to 10.1%. Too much reliance, however, cannot be placed upon these figures, as the original materials for analysis were not uniform or have not been stated. The ash is stated to consist of phosphates, sulphates, silicates, chlorides and potassium, sodium, magnesium, and calcium; potassium phosphate constituting the largest proportion.

**Nitrogenous Constituents.**—Mostly proteins; Schlossberger extracted with potassium hydroxide a substance containing 13.9% nitrogen, Mulder obtained with dilute acetic acid a substance with 16% nitrogen, and Nägeli and Loew found in a bottom yeast with 8% nitrogen as follows: Albumin, 36%; glutin-casein, 9%; peptones, 2%. The nuclein bodies, forming the main constituents of the cell nucleus, have been studied closely and were isolated by Kossel, and their presence proved by Hoppe-Seyler. Stutzer found in a beer yeast having 8.65% nitrogen, 2.26% present as nuclein.

The protein-like substance formed by the action of dilute alkalis upon nuclein resists the action of pepsin and trypsin. Other protein bodies not yet clearly defined are thought to form a gelatinous network around the cells; these are also partly transferred to the beer and aid in the retaining of larger amounts of carbonic dioxide. Reichard maintains that these gelatinous bodies are indispensable for the production of a fine, creamy foam in beer.

**Fat.**—The fat content fluctuates with the nutrition of the yeast. Nägeli and Loew state it to be about 5%. It consists, according to Darexy and Gérard, mainly of stearic and palmitic acids and a little butyric acid, partly as glycerides, partly free. It serves as a reserve food material. Lecithin and cholesterol have also been isolated from yeast by Hoppe-Seyler.

**Carbohydrates of Yeast.**—

The following have been isolated:

1. Glycogen.
2. Yeast-pectose.
3. Yeast-cellulose.

Yeast-glycogen was first obtained by Cremer (1894), who proved it to be identical with the glycogen of the liver. The dry substance of yeast contains between 31 and 32% of glycogen; its percentage may be increased by suitable nourishment. Contrary to the claim of Laurent, glycogen cannot be absorbed and assimilated from nutrient solution by yeast. Henneberg asserts that the various types of yeast can be distinguished by the extent of glycogen formation.

According to investigations made by Cremer, and later confirmed by Buchner and Rapp, yeast also contains an enzyme capable of converting glycogen into a glucose. "Yeast gum" or yeast pectinous substances have been isolated by several investigators.

Yeast cellulose or substances resembling cellulose are contained in the membrane of the cell. This cellulose behaves differently from ordinary pure cellulose; is insoluble in ammoniacal cupric hydroxide and gives none of the usual cellulose reactions. Salkowski obtained, by extraction with a 3% potassium hydroxide, a substance resembling cellulose, having a constitution of  $C_6H_{10}O_5$ ; boiled in water it was split into a soluble substance giving a red colour with iodine, and another insoluble jelly-like substance. The former, so-called erythro-cellulose, gave on hydrolysis only dextrose, whilst the latter resulted into achroocellulose and a small amount of mannose.

Payen states cellulose to be present in dry yeast up to 29.4%. Liebig and Pasteur found only 16 to 18%.

**Tannin.**—Jørgensen claims the presence of tannin in yeast during the first stages of fermentation, but Naumann and Will were not able to find it.

**Mineral Constituents of Ash.**—There are many analytical data by different authorities regarding the ash constituents of yeast and they generally are found to range between the following limits:

Potassium oxide ( $K_2O$ )	Sodium oxide ( $Na_2O$ )
23.3 to 39.5	0.5 to 2.5
Magnesium oxide ( $MgO$ )	Calcium oxide ( $CaO$ )
4.1 to 6.5	1.0 to 7.6
Phosphoric oxide ( $P_2O_5$ )	Sulphur tri-oxide ( $SO_3$ )
44.8 to 59.4	0.3 to 6.4
Silica ( $SiO_2$ )	Chlorine
0.9 to 1.9	0.03 to 0.1

**Vitality of Yeast.**—According to Hansen, yeasts retain their vitality longest in a 10% sucrose solution. Of 44 species after 20 years' observation only 3 varieties died in this solution. They die more quickly in wort, also in water, but generally keep for a period of several months to years. Drying in a very finely divided state kills yeast after a few days; some varieties may live for several months; spores are more resistant. Dried on filter paper or cotton, yeast may retain its vitality for at least one year; spores two or three years. Will made thorough and successful experiments by drying yeast with powdered wood charcoal; the yeast was still alive after 10 years.

**Heat.**—Moist heat is detrimental to yeast, and kills it between 50 and 60°; spores are more resistant. In a wine with 6.4% alcohol the yeast cells were killed after heating at 45° for 2 hours. Cooling to -130° and freezing for months is not detrimental to the yeast cells.

**Light.**—Diffused daylight and electric arc light retard the budding; sunlight kills the cells. It is not known whether yeasts also participate in the detrimental action of sunlight upon the taste and odour of beer.

**Characterisation of Saccharomycetes.**—If brought into saccharine fermentable solutions, yeast will form a sediment which increases with the period of fermentation; in breweries and distilleries this consists mostly of round or oval cells. Such yeasts are classed as the *Saccharomyces cerevisiæ* type.

*Wine yeasts* and some other types are elliptical, and are classed, according to Rees, as the *Ellipsoideus* type. A third type, called *Pastorianus*, is characterised by its elongated sausage-shaped form. These latter, *Pastorianus* yeasts, are generally detrimental to the fermentation process and are considered as disease ferments.

Although the cells of one type are not always strictly uniform,

there exist for the most part a larger number of characteristic cells enabling positive identification to be effected.

**Spore Formation.**—Besides vegetative propagation by budding, the *Saccharomycetes* cells also form endospores, the cell being transformed into an ascus. Hansen has used the different spore formation and its properties to differentiate between the various types of culture and wild yeasts. The spores of culture yeasts appear to be empty, whilst the spores of wild yeast are strongly refractive. These phenomena are utilised in the analysis of brewery yeast.

**Enzymes.**—The yeast-cell contains many enzymes distinct from each other in their respective action. The kind and number of enzymes in different types differ materially and may be considered as one of the safest and most constant factors of identification and differentiation.

Some of these enzymes have the faculty of diffusing through the cell membrane; others are partly incapable of diffusion and are then utilised for assimilation and disassimilation within the cell. Hahn proposes for these enzymes the name "endoenzymes."

A synthetic action of yeast enzymes has only been proved for "yeast glucase" by Croft Hill and Emmerling, the final product from dextrose being maltose, according to Croft Hill, and isomaltose, according to Emmerling.

The yeast enzymes may be grouped as follows:

1. *Hydrolysing enzymes:*

(a) *Sugar splitting:*

Invertase, Maltase, Lactase, Melibiase, Raffinase, Trehalase, Diastase and a glycogen-splitting enzyme.

(b) *Proteolytic.*—Endotryptase.

(c) *Coagulating.*—Rennet.

2. *Oxidising:* Oxydase, Catalase.

3. *Reducing enzymes.*

4. *Fermenting enzymes:* Zymase.

**Invertase** splits sucrose into dextrose and lævulose; raffinose is broken up into lævulose and melibiose (Bau). Invertase was first isolated by Berthelot. It occurs in brewery and other culture yeasts, as well as in most wild yeasts, is easily soluble in water, thereby differing from other sugar-splitting enzymes, acts only in acid solution and is not affected by drying for 1 hour at 140° to 150°.

**Maltase** changes maltose into dextrose; is difficultly soluble in water and can only be extracted from crushed and ground cells by leaching; it occurs in most yeast types; optimum temperature (Lindner and Kroeber), 40°. It is destroyed, according to Beyerinck, at 50° to 55°.

**Melibiose.**—This splits melibiose into dextrose and galactose; it is soluble in water and has been extracted from bottom fermenting yeasts by leaching dried cells with water. It occurs also in some of the top fermenting yeast types (Lindner).

**Raffinase** splits raffinose, but not sucrose. It occurs in several yeasts.

**Lactase** splits lactose into *d*-galactose and dextrose and occurs in only a few saccharomycetes, never in brewery culture yeast. It has been found in Kefir organisms; it does not diffuse or penetrate the cell wall.

**Trehalase** splits trehalose and is not diffusing. E. Fischer proved its presence in the Froberg type, and considers it identical with diastase; Effront does not agree with this view.

**Glycogen-splitting Enzyme.**—This was found in the yeast juice obtained by E. Buchner under high pressure. It ferments glycogen which is *not* accomplished by the yeast proper. It probably plays an important part in the "so-called" *self-fermentation or auto-digestion*. Wroblewski considers it identical with diastase.

**Diastase.**—Starch is also attacked (Wroblewski) by yeast juice in a small degree, while the yeast proper has no action upon the same. Lately yeasts have been discovered capable of fermenting dextrans.

**Proteolytic Enzyme.**—The presence of this enzyme was proved by Will, Wehmer, and Beyerinck. Beyerinck considers it similar to trypsin, as proteolysis is stronger in alkaline than in acid gelatin. Hahn, however, claims that it must act in an acid solution; its optimum action occurs in 0.2% solution of hydrochloric acid (similar to pepsin). Yeast juice proteins lose their power of coagulation after 10 to 14 days' auto-digestion. The products are tyrosin, leucin, xanthin bodies, passive albumoses, but no peptones.

Hahn and Geret have established the following properties of yeast-endotryptase; it is precipitated from yeast juice by alcohol; cannot be separated from invertase; gives no reaction with Millon's reagent. Optimum temperature, 40° to 45°, destroyed at 60°; retains its efficiency in yeast juice 9 to 15 days at 37°. Endotryptase plays

an important rôle in the auto-digestion, or self-fermentation, of the yeast.

**Coagulating Enzymes.**—The presence of a coagulating enzyme in yeast juice was proved by Rapp and in extracts obtained by treating yeast with chloroform under pressure at 60°. It coagulates boiled milk, acts towards alkalies, acids and salts as rennet, and is destroyed in solution by heating for 2 hours at 65°; very resistant when dry, remains efficient in juice for months; does not dialyse.

**Oxidising Ferments.**—Effront first presumed the presence of such an enzyme in yeast, as heat is generated when air is passed through finely-ground yeasts and yeast juice. Gruess established its oxidising action upon tetramethyl-1-4-diaminobenzene (violet); alcohol weakens it; heat (60° to 65°) destroys its action.

According to Loew's investigations, there also exists in yeast the enzyme called *catalase*, which he claims to be capable of decomposing hydrogen peroxide with the formation of oxygen.

The presence of *reducing enzymes* is indicated in yeast juice by the generation of nitrogen from nitrites, of hydrogen sulphide from sulphur and thiosulphates, as well as the reduction of iodine to hydriodic acid. The optimum temperature is 40°. The reduction of methylene blue especially, is of an enzymic character.

**Fermenting Enzyme.**—E. Buchner first showed (1897) the existence of an enzyme capable of splitting sugar into alcohol and carbonic-acid gas, and gave it the name "Zymase."

Zymase is contained, in addition to the other enzymes mentioned, in the juice obtained by means of hydraulic pressure from the yeast previously ground in admixture with quartz and infusorial earth; the zymase can also be extracted by water or glycerol from yeast that is previously killed with ether or acetone and then finely ground. Bottom yeasts are generally more suitable than top yeasts for the production of zymase.

The yeast juice ferments: dextrose, fructose, maltose, sucrose quickly, raffinose slowly, glycogen and starch very slowly, galactose very little. Lactose, arabinose and mannose are not fermented.

The zymase does not dialyse and its active power is destroyed in the juice at 40° to 50°; it acts much more slowly than other enzymes; it is precipitated by alcohol and ether together with other substances. It may be evaporated to dryness at low temperature without materially injuring its efficiency: the residue may be heated to 85° for 8

hours without harm; it may be preserved for 1 year without losing its fermentative energy. Its deterioration in the juice is due to the presence of endotryptase.

The action of zymase is increased by weak alkalies, such as potassium carbonate or sodium hydrogen phosphate. A temperature of 28 to 30° causes the quickest action, but the highest fermenting power is attained at 12° to 14°. In 30 to 40% sugar solutions zymase produces the largest percentage of carbon dioxide, but the speed of fermentation is highest in 10 to 15% solutions. Thirty to 40% sugar solutions generate 0.8 grm. of carbon dioxide within 96 hours. The yeast juice retains its fermentative power even in a dilution of 1.25.

Antiseptics do not materially influence the action of zymase; it may be preserved without injury by toluene, chloroform, sugar and glycerol. The proportions of carbon dioxide and alcohol produced by fermentation are approximately equal; succinic acid and glycerol are apparently not formed. Up to the present, zymase has never been obtained in the pure state; a preparation of active fermentative power may be made, according to Buchner, Albert, and Rapp, from "preserved yeast" ("Dauerhefe"), made by bringing yeast into ether or acetone or by heating yeast in a stream of hydrogen. Such yeast finely ground with sand can be used for the production of powerful fermentative agents. The percentage of zymase in yeast varies; it increases if the yeast is stored at low temperatures.

**Variation of *Saccharomycetes*.**—The practical application of Hansen's pure culture system is based upon the assumption that pure culture yeast does not suffer any physical changes in practice. At times changes have been observed in such yeasts, which were only temporary, but Hansen succeeded in cultivating varieties and types with permanent characteristic properties.

**The Circulation of Yeast in Nature.**—The normal source and origin of yeast are the damaged surfaces of sweet juicy fruits, the juice of which forms the natural and best nutrient for their propagation. Rain washes the yeasts to the ground, where they remain during winter and spring, when they are again transferred to their summer breeding places. Insects are active factors of transferring and distributing the yeast cells. Soils of orchards are especially rich in yeasts.

**Important Yeast Types of the Brewing, Distilling and Wine Industries.**—Culture yeasts are such as have been cultivated for

long years in the fermentation industries and possess certain qualities which make them especially adapted and available.

## CULTURE YEASTS

The following are those most frequently mentioned in literature:

1. *Saccharomyces Cerevisiæ*.—Hansen, from English and Scotch breweries; a vigorous beer top yeast.
2. *Carlsberg Bottom Yeast*, 1.—Hansen.
3. *Carlsberg Bottom Yeast*, 2.—Hansen.  
No. 1 produces very stable beer, not so readily clarifying.  
No. 2 beers not so stable, but with better clarification.
4. *Four Culture Yeasts* from Munich Station (described by Will). Tribes 93, 2, 6 and 7; the first two having a high fermenting power, tribe 6 with a medium, and tribe 7 with a low fermenting power.
5. *Distillery Yeast*, II, Berlin, isolated from a distillery in West Prussia, is a top yeast of the Froberg type, suited for fermenting highly concentrated mash, difficult to ferment and possessing great power of resistance to high alcoholic content.
6. *Berlin Race*, V, mostly used for the manufacture of compressed yeast.
7. *Wild Yeast*.—*Saccharomyces Pastorianus*, I, II, III.—Hansen. Sausage-shaped cells, disease ferments in beer. I imparts a bitter taste and odor; III causes turbidity. I may give a good product in the preparation of wine. All occur in air. II and III are top yeasts, I is a bottom yeast.
8. *Saccharomyces ellipsoideus*, I and II.—Hansen.  
Yeast I.—A wine yeast, found by Hansen on the surface of ripe grapes in the Vosges district, cells have an ellipsoidal shape; found to be useful and active in wine fermentation.  
Yeast II is a dangerous disease yeast for breweries causing turbidity. Two similar types have been isolated by Will.
9. *Saccharomyces ilicis* (bottom yeast) and *S. aquifolii* Grönlund (top yeast) found on fruit of *Ilex aquifolium*. They produce bitter and disagreeable taste in worts; cells mostly spherical in shape.

10. *Saccharomyces pyriformis*.—Marshall Ward. Produces alcoholic fermentation of English ginger beer, forms together with *Bacterium veriforme* the so-called gingerbeer plant, used for the production of an acid frothing beverage—ginger beer.
11. *Saccharomyces membranæfaciens*.—Hansen. Found in wines, also in polluted waters; generates from sugar no alcohol, but acids; propagates in the presence of 12% alcohol; consumes malic, acetic and succinic acids; destroys the bouquet of wine.
12. *Saccharomyces mali*, Du Clauxi, Kayser, isolated from cider; ferments invert sugar, and produces esters (bouquet).
13. *Schizosaccharomyces pombe*, found by Saare in pombe (negro millet beer) from Africa. A top yeast, also fermenting dextrin; used in South American distilleries with success.
14. *Schizosaccharomyces mellacci*.—Jørgensen. Isolated from Jamaica rum mash. Greg claims to have found 8 similar species in Jamaica rum mashes.
15. *Schizosaccharomyces octosporus*.—Beyerinck. Found on currants and raisins (Greece); ferments maltose and dextrose, but not saccharose, shows characteristic ascus formation.

## PURE CULTURE OF YEAST AND ITS APPLICATION IN PRACTICE

Pasteur showed that bacteria could cause disease and detrimental effects in these industries, and that one of the principal sources of infection in the brewery was the open *coolship*. His proposal to replace the coolsh p by closed apparatus was not universally approved and did not find any practical recognition. The reason is that this method does not prevent beer diseases, as another and very dangerous source of infection still exists, namely, the employment of impure yeast.

Pasteur had advocated a cleaning of yeast by means of tartaric acid, which kills part of the bacteria, but, at that time, he did not know of the existence of the abnormal or disease yeasts and, as Hansen proved later, the addition of tartaric acid favoured the development of these disease yeasts.

Hansen proved, in 1879, that numerous abnormal phenomena were directly caused by yeasts which are contained in the pitching yeast, besides culture yeasts. He showed that the culture yeast does not

consist of a *uniform* species, but of many varieties, of which each one imparts to beer peculiar properties and which employed collectively may at times even cause disease phenomena. Actuated by these investigations, Hansen founded his system of the use of pure culture yeasts in breweries, based upon the fact that by systematic selection a single suitable type may be made from the culture yeast which is *alone* allowed to develop in the wort.

**The pure cultivation of yeast** is made by the following method: a suitable quality of yeast is mixed with sterile water, and a small quantity of this mixture is distributed into wort gelatin so that the various cells are separated from each other on cover-glasses. These cover-glasses are then transferred to small moist chambers and the development of one cell is continually observed under the microscope. After these colonies are large enough a part of them is carefully transferred into sterile wort for further propagation. In this manner an absolutely pure culture is obtained from a single cell; these cells are examined as to their action in wort and the most suitable or appropriate species are selected for use in practice. Hansen showed that the properties of the yeasts in practice are not subject to variation in a serious degree, and that some varieties quickly assume their old characteristics.

The so-procured culture yeast is then transferred to larger quantities of a nutrient wort, and, in a short time, sufficient yeast is propagated for a large quantity of wort. The time and period of stability and purity of such a cultivated yeast differs according to general conditions, seasons, etc., and the resisting powers of the different pure culture species differ considerably.

It is, therefore, necessary to introduce new pure-culture yeast periodically into a brewery; as soon as the biological tests and control show any deterioration of the pitching yeast, it should be renewed. The production of large quantities of pure cultivated yeast is accomplished in the so-called pure-culture apparatus in breweries and institutes devoted to fermentation industry in the manner described by Hansen.

The apparatus provides for aeration of the sterilised wort with filtered air; the wort is continually fermented and the yeast sediment is retained. Hansen introduced personally the production and application of pure-culture yeast in bottom fermenting breweries, and the largest plants in the world work according to his system,

which has abolished all empiricism and replaced it by absolutely safe working methods in practice. The pure culture of top fermenting yeast was first applied in practice by Jørgensen in 1885.

**Spirit and Compressed Yeast.**—The pure-culture system has also found recognition by these industries owing to the efforts of Prof. Lindner in Berlin. He has introduced Race II for almost all distilleries in Germany with good results. In the United States they are only used to a limited extent.

Recently, pure cultures of lactic acid bacteria have been used together with pure-culture yeast in the distilling industry in order to prevent the harmful butyric acid fermentation. The pure culture has also been used for the manufacture of compressed yeast, particularly by the efforts of Lindner who recommended Race V for this industry.

#### PURE CULTURE IN WINE INDUSTRY

This has been introduced especially since Wortmann has proved by his searching investigations that different wine-yeast types are capable of yielding most different products in regard to acidity, bouquet, as well as taste. It was also expected that yeasts from different localities would impart to any must some characteristics, but it is found that the taste and bouquet are dependent to a much larger degree upon the type of grapes, the soil, degree of ripeness, and some other factors. The bouquet substance and flavor of yeast are of a volatile nature; the use of foreign wine-yeast types has, therefore, been abandoned, and pure cultures are mostly made from the yeast types found on the native grapes and in the produced wines.

The main advantage of pure culture in wine manufacture is the rapid and vigorous fermentation before the foreign germs and wild yeasts, especially *apiculatus* or the equally dangerous acetic-acid bacteria, are capable of any vigorous development. Pure-culture wines also clarify more quickly and better, and the bouquet of the young wines is generally purer.

Owing to the extremely short season of wine fermentation and the great variability of yeast types for the wine manufacture, the pure cultures are not cultivated in large apparatus as in the brewing industry, but the yeasts are fermented in small quantities by

establishments specially devoted to this work. The wine producer propagates this yeast in about 2.5 to 3 gallons (10 to 12 litres) of pure boiled must and adds this to the bulk of his must as soon as it is in a vigorous stage of fermentation.

Very good results have been obtained in the manufacture of sparkling wines by Wortmann, who isolated races having the characteristic property of forming a solid sediment on the cork and producing but very little turbidity.

Seifert has introduced the pure culture especially for production of sweet wines, which are distinguished by their resistance towards high concentrations of sugar and alcohol.

#### CIDER MANUFACTURE

In the production of this beverage pure cultures have already been introduced successfully by Wortmann, and Kramer. Jörgensen, Kayser and Nathan have investigated their use in this field and recommend the same procedure as in the wine manufacture. The results have been satisfactory, the yeasts imparting to the cider a more or less vinous taste and flavour.

#### YEAST IN BAKING

*Saccharomyces cerevisiæ* generates in the dough carbon dioxide and alcohol from the dextrose and maltose formed in bread during the raising and baking process. The alcohol assists the carbon dioxide in its power of raising the dough, causing the sponginess of bread, owing to the fact that it is mostly volatilised in baking. Top fermenting yeast was mostly used formerly; the bottom fermenting yeast is very slow in action and bitter; both kinds of yeast have now been entirely replaced by the so-called "compressed yeast."

The yeast mash forms an abundant yeast foam which is skimmed off, washed, watered and deprived of most of its water by filter presses or centrifuges.

**Air yeast** is produced from yeast mash in large fermenting vats by aerating it with sterilised air. After a fermentation of 20 hours, the yeast is washed and pressed in filter presses.

**Good compressed yeast** is of a light, pale yellow color, somewhat crumbly, not slimy, and of a pleasant odour. It must be protected

from light and air, and kept at low temperatures; it should be even in texture, should have no sourness, an apple or fruity, not cheesy, odour, and should not exhibit any dark specks and streaks. It is sometimes mixed with starch, but this is not necessary, as the machinery of to-day removes the water sufficiently. The former occasional addition of gypsum and chalk as adulteration is hardly met with to-day.

At times bottom yeast from which the bitter taste has been removed is added to compressed yeast; this may be recognised by the absence of spore colonies and the remnants of organic hop particles, especially the lupulin or oil glands of hops.

Authorities seem to differ in regard to the addition of starch, but most of them state that starch is added for the purpose of reducing cost, and consider it a reduction of quality and a distinct adulteration. The addition of starch should be stated on the label.

Compressed yeast should be used when fresh; it easily becomes stale and deteriorates; it is generally wrapped in tinfoil and kept cold; texture and fracture should be uniform and even.

**Compressed Yeast—Keeping Qualities.**—The *keeping qualities* of compressed yeast can be ascertained by incubating the yeast for a period of two to three days at a temperature of 25 to 30°. During this time and at the end of incubation, the yeast should not become soft and show any signs of decomposition or incipient putrefaction, as indicated by a stale or cheesy odour, or a darkening in colour.

A microscopical examination will also aid in determining the soundness and vitality of the yeast, by estimating the amount of dead cells. The latter can be made readily discernible in the microscopical specimen by employing the staining method with methylene-blue, as described by Schlichting and Winther in a paper read before the International Congress of Applied Chemistry, 1912. A good healthy compressed yeast should contain no more than 3 to 5% of dead cells.

**Method for Detecting Mycoderma.**—The presence of budding fungi of the mycoderma group can be ascertained by a microscopical examination. More definite results are shown by mixing the yeast in wort gelatine and pouring this mixture into Petri dishes, which are then incubated at a temperature of 20°. After observation for a period of three to four days, mycoderma, if present, will form flat,

film-like colonies, spreading over the gelatine surface. A microscopical examination of the colonies will serve to identify the nature of these organisms.

**Compressed Yeast—Baking Test** (Koenig, *Chemie Nahr. u. Genussm.*, 1914, 3, 694, part 2).—In order to determine the relative fitness or raising period of compressed yeast for baking purposes, it is necessary to conduct miniature baking tests, which can be carried out in the following manner:

Prepare a dough from 280 grm. of wheat flour, 160 c.c. of a 5% salt solution, and 2 grm. of pure cane sugar. While mixing these ingredients, add 10 grm. of the compressed yeast to be tested. All the ingredients should have a temperature of approximately 30°. The total time consumed for mixing the dough should be 10 minutes.

The dough is dropped into a baking pan of exact dimensions, *i.e.*, bottom 14 × 9 cm., top 15 × 10 cm., depth 8.4 cm. Care should be taken that the dough is rolled to identical thickness and shape. A metal rod is laid over the top of the pan and the dough is then incubated in a thermostat which is kept at 33 to 35°.

The "raising period" is represented by the total number of minutes consumed, figuring from the beginning of mixing the dough until the time when the metal rod is touched by the raised dough.

**Dry yeast** is classed by Leach as a product obtained by mixing fresh yeast with starch or meal into a stiff dough which is subsequently dried at low temperature and under reduced pressure; this preparation is said to keep for a long time, and although the cells are largely rendered inactive by the drying process, they do not lose their power of fermentation.

## PHYSICAL EXAMINATION OF YEAST

Examination of the physical characteristics of yeast is often made by the chemist, and is no doubt of some practical value, although the results cannot be claimed as decisive. Among the more important physical tests the following may be mentioned:

1. The yeast should form a solid sediment after fermentation.
2. The yeast should be crumbly, not doughy or slimy.
3. Odour should be pleasant, pure, clean, aromatic, not sharp, repulsive, or cheesy, taste slightly and agreeably bitter.

4. Colour should be light yellow (sometimes darker from beers with deeper colour).
5. Mixed with cold water it should settle rapidly and form a solid compact sediment.

For a more extensive and comprehensive examination we must resort to a microscopical and biological examination.

#### MICROSCOPICAL EXAMINATION

Some of the sample is mixed with sterilised water to a milky fluid; some of this is brought by means of a sterile glass rod upon a slide, the cover-glass put on and the yeast examined under a magnification of 600 to 800. Thus the form, shape and size of the cells are observed, the condition of the cell membrane and the cell contents (protoplasm); the presence of *dead yeast* cells is determined by adding to another sample of the water and yeast mixture a drop of stain, such as methyl violet, eosin, or fuchsin. A solution of the stain is made by dissolving 1 grm. of the dye in 160 c.c. of water and 1 c.c. of alcohol. Living and active cells do not absorb the stain so readily, whilst dead cells are immediately stained.

Lindner recommends the following stain and method: 1 part of powdered indigo is rubbed with 4 parts of concentrated sulphuric acid, allowed to stand 24 hours, diluted with 20 to 30 times its volume of water, heated to 50° and neutralised with calcium or sodium carbonate. To a sample of yeast 1 drop of staining solution is added, allowed to act a few seconds, diluted with a weak sucrose solution, the whole thoroughly mixed and a drop examined on the slide under a cover-glass. Old and dead yeast cells exhibit a thickened membrane. A good yeast should contain no more than 3 to 4% of stained cells.

The microscope field shows also the presence of foreign ferments, the addition of other substances, such as starch, as well as contamination with mould spores and bacteria. The detection of the latter is simplified by adding a 5% sodium hydroxide solution to the preparation.

It is obvious that a yeast should be as free as possible from any contaminating, foreign organisms, such as lactic, acetic, and butyric acid bacteria. It should also be free from any so-called wild or abnormal yeasts (as described below). These are generally recog-

nised by their variation and distinct difference in shape, although the presence of abnormal yeasts (not culture yeasts) should always be corroborated by the so-called plate cultures and spore methods as described in special works by Hansen and Klöcker.

The formation of ascospores as studied by Hansen proceeds under the following prevailing conditions:

1. Abundant air must be admitted to the yeast cells; propagation occurs on a moist surface (gypsum block).
2. Only young, vigorous cells produce these spores.
3. The optimum temperature is 25° for the most known species.
4. Some few species form spores, even if present in fermenting, nutrient solutions.

The fundamental differences between the various species are especially the temperature and the time necessary for spore formation, *i. e.*, *S. cerevisiae* is distinguished from the wild yeasts in that the latter form ascospores in a much shorter time under the same conditions and temperature. The ascospores of the culture yeast are also much larger than those of wild yeasts.

#### CHEMICAL TESTING OF YEAST

The water and ash of a yeast are determined according to the standard methods of food analysis.

The general and most important criterion for the valuation of yeast in the fermentation industry is the so-called fermentative and raising power which is especially required for the valuation of compressed yeast.

By activity or energy of fermentation is understood the degree of intensity with which a yeast is able to decompose a certain quantity of sugar within a specified time. It not only serves to distinguish the various yeast species from each other, but is also useful in establishing a criterion for the different physiological conditions of any given species of yeast.

The methods used for this determination are based upon the estimation of the carbon dioxide generated from a sugar solution. The amount is ascertained either by weight or by volume. The former method, according to Meissl, is especially applicable if only small amounts of yeast are at disposal. The latter (Hayduck and Kuss-erow) is principally used to examine yeasts in practice.

**Meissl's Method.**—This method determines the weight of carbon dioxide which is generated by 1 grm. of yeast within 6 hours at 30° from a solution in ordinary tap water of a mixture of 400 grm. of pure sucrose, 25 grm. of ammonium phosphate and 25 grm. of potassium phosphate.

According to Meissl, a "normal" or "standard" yeast is one that liberates under these conditions 1.75 grm. of carbon dioxide, and the energy of the yeast is then returned as 100.

The fermenting power is then found according to the following equation:

$$1.75 : n = 100 : x \text{ in which}$$

$$n = \text{quantity of carbon dioxide.}$$

Prior found, according to this method, the following values for the various species:

Carlsberg bottom yeast, No. 1,	136.40
Carlsberg bottom yeast, No. 2,	106.13
<i>S. pastorianus</i> I,	155.48
II,	280.72
III,	202.20
<i>S. ellipsoideus</i> , I,	285.76
II,	219.03

#### FERMENTING POWER

**Meissl's Method.**—Of the above mixture 4.5 grm. are dissolved in 50 c.c. of tap water; it is also suggested to use gypsum water by mixing 15 parts of a saturated solution of calcium sulphate with 35 parts of distilled, aerated water.

The solution is introduced into an Erlenmeyer flask of about 100 c.c. capacity, together with exactly 1 grm. of yeast, which should be thoroughly distributed so as to form a uniform mixture without lumps. The flask is then fitted with a doubly perforated rubber stopper, having 2 tubes, one of which is bent and passes nearly to the bottom of the flask and is fitted at the other end with a rubber tube and glass plug, whilst the other is connected with a calcium chloride tube. The whole apparatus thus arranged is weighed accurately and kept in a thermostat or water-bath at 30° for 6

hours. At the end of this time it is removed from the thermostat, quickly cooled in cold water, the rubber tube and glass plug taken off and the remaining carbon dioxide drawn out by suction; the glass plug and rubber tube are replaced and the flask carefully weighed as before. The loss of weight is equal to the quantity of carbon dioxide generated by the fermentation of the sugar owing to the activity of the yeast. A good compressed yeast should have at least 75 to 80% of fermentative energy or fermenting power.

**Methods of Hayduck and Kusserow.**—In both methods the carbon dioxide generated by a given quantity of yeast in a certain sugar solution is measured by volume. Hayduck measures the volume of carbon dioxide directly, whilst Kusserow ascertains the quantity of water displaced by it and measured in a graduated cylinder, which serves as a receptacle.

Hayduck uses an apparatus similar to Scheibler's carbon dioxide apparatus, consisting essentially of a 500 c.c. burette divided into c.c. and connected by a rubber tube with a large glass bulb.

For both methods the following procedure is prescribed: 40 gramm. of pure sucrose are dissolved in 400 c.c. of water and this solution brought to 30°. 10 gramm. of compressed yeast are intimately mixed with successive portions of the solution in a porcelain dish until no more lumps are visible. The mixture is introduced into a 1000 c.c. flask and the porcelain dish is thoroughly rinsed with the remainder of the sugar solution until all of the solution is put into the flask; the whole is now thoroughly shaken and placed into a water-bath at 30°, allowed to stand open in the bath for 1 hour, while carbon dioxide freely escapes.

The flask is then connected by means of glass and rubber tubing with the apparatus filled with water to the zero mark. In order to avoid any absorption of carbon dioxide by the water, a little petroleum is used floating in a very thin layer upon the water.

After  $\frac{1}{2}$ -hour's connection the vent is closed, the flow of carbon dioxide is shut off, and the measured volume of gas is read on the burette after the water in the burette and the glass bulb have been brought to an equal level. The c.c. of carbon dioxide may express directly the fermenting power of the yeast; or the weight of sucrose, which has been decomposed by 100 gramm. of yeast, may be calculated by multiplying the figure found by 0.03841; as 342 gramm. of sucrose will give 176 gramm. carbon dioxide, 1 c.c. of which weighs

0.001977 grm. The weight of sucrose necessary for the production of 1 c.c. carbon dioxide =

$$\frac{342}{176} \times 0.001977 = 0.003841.$$

**Kusserow's Method.**—In this method exactly the same quantities of materials are used, but after the flask has been standing in the water-bath at 30° for an hour, it is connected with another flask of exactly the same capacity absolutely full of water. This flask has a doubly perforated stopper through which pass two glass tubes, both bent at right angles, going to the bottom of the flask, and bent again at right angles, under the flask is a graduated cylinder of 500 c.c.

After an hour the gas is allowed to pass into the second flask; it forces the water out and this is collected in the graduated cylinder.

A good baker's yeast should have according to this method a fermenting power of 250 c.c.

Kusserow also estimated the fermenting power after the first and second half-hour; during the first period a good yeast should give 50 c.c. and in the second half-hour 150 c.c. of carbon dioxide.

**Acidity.**—5 to 6 grm. of yeast are mixed with distilled water, introduced into a flask and titrated with sodium hydroxide  $\frac{N}{1}$ , phenolphthalein being used as an indicator. The acid degree is expressed in mg. of the alkali for 100 grm. of yeast or in % of lactic acid; 1 c.c. normal alkali = 0.09 grm. lactic acid.

**Starch.**—The addition of starch to yeast before pressing has long been customary, basing its use upon the drying qualities of starch. The best grades of compressed yeast contain about 5%, some as high as 50%. The larger amounts are looked upon as an adulteration.

**Estimation of Starch.**—See under starch.

**Medicinal Use of Yeast.**—The therapeutic value of yeast, which has been recognized for a number of years, is chiefly dependent upon its vitamin content, as well as other important constituents such as nuclein, various enzymes of an oxidizing and reducing nature. It has been shown that yeast exerts an inhibiting influence or neutralizing effect upon the toxins produced by various pathogenic organisms, such as cholera, typhus, and diphtheria bacilli, as well as streptococci and staphylococci.

C. Funk (*British Medical Journal*, 1913, 1, 814) found that vitamins isolated from yeast and injected into pigeons suffering from polyneuritis, completely restored them to a normal condition.

Similar results were found by A. Seidell (*Public Health Reports*, 1916, 31, 364-70). P. B. Hawk, et al. (*Journal Amer. Medical Assn.*, 1917, 1243-47) found that yeast exerts a decided curative effect in the treatment of skin diseases. It was also shown by these investigators that yeast can be used with beneficial effect in diseases of the gastro-intestinal tract.

K. Schweizer (*Chem. Zeitung*, 1919, 33-36, 69-72) states that yeast dried below 100° forms a valuable remedy against beri-beri and kindred diseases.

The analysis of medicinal preparations containing yeast should be carefully conducted with the aid of standard chemical, microscopical, as well as biological methods.

**Yeast Extract as Substitute for Meat Extract.**—Yeast has been used extensively in late years for the preparation of certain alimentary products, more specifically as substitute for meat extracts (see Vol. VIII).

Various processes are employed in the manufacture of yeast extract. However, the majority of these processes depend mainly upon a preliminary autodigestion of the pressed yeast. Many patents have been granted for these processes, one of which may be briefly summarized: English Patent 134168-30-4-19 J. J. C. A. and F. M. O'Sullivan, Wolsey Bridge, Staffs and M. L. Marsan, Paris:

"Pressed yeast is allowed to undergo autolysis after which it is introduced into boiling water, and subjected to two boilings and skimmings. It is finally concentrated in an open, jacketed pan under constant skimming until the product sets to a semi-solid mass on cooling."

The distinguishing mark of true meat extracts is their content of *creatine* and *creatinine* which are entirely absent in yeast extract. (For complete analytical and tabulated results, see Allen, Vol. VIII.)

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Hansen, E. Chr. *Practical Studies in Fermentation.*

- Jago. *Science and Art of Breadmaking.*  
Jørgensen. *Micro-Organisms of Fermentation.*  
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Koenig. *Chemie der Nahrungs und Genussmittel.*  
Koenig. *Unters. landw. und gewerbl. wichtiger Stoffe.*  
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# NEUTRAL ALCOHOLIC DERIVATIVES

HENRY LEFFMANN

Under this title will be included a number of important substances, such as ether, chloroform, chloral, formaldehyde and several esters. Those members of the group that are used as general anesthetics have been submitted to an extended examination by Baskerville and Hamor (*Jour. Ind. Eng. Chem.*, 1911, **3**, 301; *ib.*, 1912, **4**, 212, 278, 362, 422, 499, 571; *ib.*, 1913, **5**, 828) and many of the data here given have been taken from their papers.

**Ethyl Ether. Ethyl Oxide.**—When used without qualification, the term “ether” always refers to this substance. Being prepared by the action of sulphuric acid on alcohol, it has received the erroneous, but often used, title of “sulphuric ether,” a name that properly belongs to ethyl sulphate. The crude product contains several impurities, among which are alcohol, water, sulphurous and acetic acids and fusel oil. By addition of water much of the alcohol may be eliminated, the ether forming a layer on the surface. The acids and most of the water may be removed by agitation with potassium carbonate, and the ether obtained nearly pure by redistillation.

Ether is a colourless, limpid, mobile liquid with a characteristic odour and a sharp, pungent taste. It is very volatile, and although it is one of the lightest of liquids, the vapour is one of the heaviest of the common gases. Both liquid and vapour are highly inflammable. Mixtures of ether vapour and air may explode when heated to 190°. The conditions of such explosions have been recently investigated by McClelland and Gill (*Scientific Proc. Roy. Dublin Soc. n. s.*, 1920, **16**, 109).

The b. p. is 34.6° and the sp. gr. 0.7178 to 0.719 at 15°/4°, but if not promptly placed in closed vessels, the sp. gr. will quickly rise to 0.720 or even to 0.721. Ether is sparingly soluble in water, less so in glycerol, which solutions are neutral to ordinary indicators. It is miscible in all proportions with alcohol, chloroform, benzene,

petroleum spirit and volatile oils. It dissolves resins, fats, many alkaloids, phosphorus, bromine, iodine, and ferric, mercuric and auric chlorides. When exposed to air it oxidises slowly to acetic acid.

*General Tests for Impurities.*—Non-volatile or slightly volatile impurities may often be detected by evaporating 25 c.c. of the sample, added in small portions, upon a 10 cm. filter paper, placed in a flat porcelain dish. Pure ether will leave no residue. The odour of a residue may often afford a clue to the nature of the impurity. If the result is doubtful, the test may be repeated with a larger volume of the sample.

Moist blue litmus paper should not be reddened when immersed in the sample, and the sample agitated with water should not impart to the latter any substance of acid reaction. 10 c.c. of the sample mixed with 0.1 c.c. N/10 potassium hydroxide should turn phenolphthalein solution red. Shaking 20 c.c. of the sample, 10 c.c. of water and 2 drops of phenolphthalein solution should produce a liquid of the same depth of tint as that obtained by treating the same amount of pure water.

The presence of sulphuric acid may be detected by saturating the ether with sodium carbonate and adding barium chloride, under the usual precautions.

Ether is liable to contain peroxides. Allen found a sample that liberated iodine from potassium iodide, and suggested that the action might be due to ethyl nitrite, but Baskerville and Hamor regard it as due to peroxides. Tests for such contaminations are given in connection with the examination of commercial ether.

**Commercial Ether.**—Commercial ether always contains water and alcohol, and may contain other impurities, by-products from the process of manufacture, or produced by exposure to air or to other contaminations. For many purposes, especially in its use as an anesthetic, a high degree of purity is required, and many methods of examination have been suggested. These have been carefully investigated by Baskerville and Hamor, and the results given in the articles quoted above.

**Water.**—One volume of water is soluble at ordinary temperatures in 35 volumes of ether. The U.S.P. defines ether as containing about 96% of ethyl oxide, 4% of alcohol and a little water. Metallic sodium removes both water and alcohol, and ether free from these

contaminations may be obtained by distillation. Of the many tests proposed for the detection of water, the following are the best.

*Copper Sulphate Test.*—1 grm. of anhydrous copper sulphate is shaken with 20 c.c. of the sample. Mere traces of water cause the salt to assume a blue tint. Alcohol does not interfere.

*Sodium Test.*—Metallic sodium serves for the examination of anesthetic ether. 15 c.c. of the sample are put in a perfectly dry test-tube and a piece of clean sodium, about 0.5 grm., added. The ether should not be coloured after the expiration of 6 hours, no flocculent precipitate or turbidity should be produced, and the coating on the sodium should be white and of small amount. Baskerville and Hamor point out, however, that sodium reacts in this test with both acetaldehyde and water.

*Calcium Carbide Test.*—This is a delicate and satisfactory test. 1 grm. of crystallised calcium carbide is added to 20 c.c. of the sample, and the escaping gas led through a solution of ammonio-silver nitrate. As the volume of acetylene produced by the reaction of the carbide on the water present is considerable, a quantitative determination may be made by measuring the volume thereof.

*Carbon Disulphide Test.*—Equal volumes of carbon disulphide and ether are mixed thoroughly. No turbidity should result. This test is regarded as satisfactory.

*Potassium Phenate Test.*—Potassium phenate is a highly hygroscopic substance, quite insoluble in anhydrous ether, but dissolving in small amount in ether containing water, and imparting thereto a reddish-brown tint. When carefully conducted this test will reveal as small an amount of water 0.25%, but not below this limit.

*Rosaniline Acetate Test.*—This serves for the detection of both water and alcohol, and is described in connection with the tests for the latter.

**Alcohol.**—Alcohol and ether being freely soluble in each other, and the former being used in the preparation of the latter, commercial ether, as stated above, may be expected to contain appreciable amounts of alcohol. Moreover, perfectly pure ether in contact with moisture will form some alcohol. Some of the tests used will show reactions in the case of other substances, and care must be taken not to misinterpret the results.

*Iodoform Test.*—This, commonly called Lieben's test, depends on the formation of iodoform, but a large number of substances allied to alcohol and ether also produce the same compound. In practice, acetaldehyde is the important one, and in applying the iodoform test, the absence of this substance should be first established. Baskerville and Hamor have materially improved the test by taking advantage of a suggestion of Classen, namely, to shake 30 c.c. of the sample with 20 c.c. of water, warming to 40° to expel any dissolved substances other than alcohol, and then applying Hagar's modification of Lieben's method as follows:

To 10 c.c. of the water, 5 or 6 drops of a 10% solution of sodium hydroxide are added, and the mixture warmed to about 50°. A solution of potassium iodide, fully saturated with iodine, is added drop by drop with agitation, until the liquid becomes permanently yellowish-brown, when it is cautiously decolorised by sodium hydroxide. In the presence of even small amounts of alcohol, a yellowish crystalline precipitate separates. The crystals are hexagonal plates and rosettes and are easily identified by the microscope.

*Rosaniline Acetate Test.*—This is a very delicate test, the manipulation and application of which was specially studied by Allen, whose procedure is as follows: A minute quantity of powdered rosaniline acetate is placed in a narrow test-tube, 10 c.c. of the ether added, the tube corked, and the whole agitated. If the ether is anhydrous, the coloration of the liquid will be almost inappreciable. If the coloration is considerable, 10 c.c. of ether treated with calcium chloride is placed in another tube of the same bore as the first, adding the dye as before. 0.1 c.c. of alcohol is then added from a finely divided burette, and the mass shaken. If this quantity of alcohol is insufficient to produce a tint equal to that of the sample, further additions of alcohol must be made until the liquids have the same depth of tint, best observed by holding the two tubes side by side in front of a window and looking through them transversely. The use of a piece of wet filter-paper behind them facilitates the observation. It is well to permit the alcohol to drop right into the ether, and not allow it to run down the sides of the tube, as in the latter case it will dissolve any adherent particles of dye, forming a solution which will be precipitated on mixing with the ether. It is also not advisable to dilute the sample with pure ether, so as to

reduce the colour to that of a standard tint. In practice, each 0.1 c.c. of alcohol added from the burette may be considered as indicating 1% of impurity in the sample; the error thus introduced is insignificant when the percentage of alcohol is small. The method is unsatisfactory when the alcohol exceeds 5% of the sample, owing to the intensity of the tint. The results are within 0.25% of the truth. Occasionally the tints of the two liquids are not readily comparable, but on placing the tubes for a few minutes in cold water, this difficulty is overcome. It has been pointed out by E. R. Squibb, that this test fails to detect less than 0.2% alcohol, but allows of the recognition of very minute traces of water.

Ether free from alcohol is soluble in eleven times its volume of water. Agitation with water extracts any alcohol it may contain, and thus diminishes the volume of the ether. With certain precautions, this method may be used in connection with the above test. The following are the details of the procedure that Allen devised: A small quantity of rosaniline acetate is placed in a separator which is then filled with water and a small proportion of ether, and the whole agitated. A coloured etherised water is obtained, in which ether is quite insoluble, whilst alcohol readily dissolves. 10 c.c. of the etherised water are run into a glass tube holding about 25 c.c., and having divisions of 0.1 c.c.; 10 c.c. of the sample of ether are next added, the tube corked, and the whole well shaken. On the ether rising to the surface, its volume can be easily read off. Any reduction in its volume is due to admixture of alcohol. Each 0.1 c.c. lost represents 1% of alcohol. If the alcohol does not exceed 20% the ether will be colourless, and the result of the experiment will be correct; but if the alcohol is much above 20% the ether will be coloured, and the result below the truth. The absence of colour, therefore, in the ethereal layer, indicates the accuracy of the experiment. If the ether is coloured, an accurate result can still be obtained by adding 5 c.c. of anhydrous ether, and again agitating. It is better, however, to dilute a fresh portion of the sample with an equal volume of pure ether, and use the diluted sample instead of the original. By proceeding in this manner the proportion of alcohol in mixtures of that liquid with ether can be ascertained within 1 or 2% with great facility. The process has been verified up to 60% of alcohol.

In all cases the proportion of alcohol must be deduced from the reduction in the volume of the ether, and not from the increase in that of the aqueous liquid. Care must be taken to prevent any volatilisation of the ether.

For the determination of small amounts of alcohol and water in ether, Mallinckrodt and Alt have devised the following process (*Jour. Ind. Eng. Chem.*, 1916, 8, 811).

The flask, shown in Fig. 2, is a 100 c.c. Regnault pyknometer, such as used for taking the density of solids. *X* is a cotton plug intended to act as a filter. 15 grm. of potassium carbonate, dried at 250°, are placed in the bulb *A*, and accurately weighed, after replacing the stem *B*. The stem is then removed, 50 c.c. of the sample introduced quickly, the stem replaced, and the apparatus allowed to stand for 14 hours with frequent shaking. The stopper *C* is removed, the flask inverted and held in the hand so that the ether can filter out through the plug *X*. Care must be taken that no suspended matter passes out with the ether, which should be allowed to stand in the receiving vessel for a short time to see that no finely divided carbonate settles. The space *B* is then filled with absolute ether, the stem replaced and the ether drawn in by pouring ether on the bulb *A* to cool it. The mixture is shaken, the ether removed through the filter as before, and this operation repeated four times. These procedures remove the alcohol from the salt. The apparatus without the stopper *C* is dried at 50° until the carbonate can be shaken down into the flask. The stem is removed, and replaced with an apparatus containing dry potassium carbonate and dried for 1 hour at 50°. The drying tube is removed, the ether vapour in the flask driven out by a gentle suction for 15 seconds, the stem replaced, the apparatus cooled in the desiccator and weighed. Care must be used to remove all the ether vapour, and at the same time not introduce moisture. It is advisable to make a second weighing after drying for another half hour. The difference between the weights of the carbonate before and after treatment with ether is the weight of water in the sample.

For the determination of alcohol, 100 grm. of the sample are shaken frequently during 14 hours with 40 grm. of freshly dried potassium carbonate. The sp. gr. of the ether is then accurately determined. The following diagram gives the alcohol content when the sp. gr. is determined at 25° on the international hydrogen scale.

Percentage of ether by weight	Specific gravity	Percentage of ether by weight	Specific gravity	Percentage of ether by weight	Specific gravity
99	0.72021	89	0.73298	79	0.74495
98	0.72152	88	0.73428	78	0.74612
97	0.72284	87	0.73547	77	0.74729
96	0.72416	86	0.73666	76	0.74846
95	0.72541	85	0.73785	75	0.74975
94	0.72666	84	0.73904	74	0.75104
93	0.72792	83	0.74022	73	0.75233
92	0.72918	82	0.74141	72	0.75362
91	0.73043	81	0.74260	71	0.75492
90	0.73168	80	0.74378	70	0.75623

**Acetone.**—Several tests have been proposed. The following seem to be the most satisfactory.

*Hydroxylamine Test.*—3 c.c. of the sample are shaken with a convenient amount of a 5% solution of hydroxylamine hydrochloride, and the mixture then agitated with 4 c.c. of sodium hypochlorite solution. In presence of acetone, a blue or bluish-green tint is imparted to the ether. Excess of sodium hypochlorite should be avoided.

*Mercuric Chloride Test.*—The Dutch Pharmacopœia recommends the following procedure: 2 c.c. of the sample are mixed with 6 drops of mercuric chloride solution and 6 c.c. of barium hydroxide solution. The mixture is filtered and the filtrate tested with ammonium sulphide. The presence of an acetone mercurial compound is shown by the production of a precipitate.

**Acetaldehyde.**—This is a not infrequent impurity of ether, and examination should be made for it. The following test is recommended:

*Potassium Hydroxide Test.*—5 gm. of potassium hydroxide in small freshly broken pieces are covered with 30 c.c. of the sample and kept in a tightly closed vessel in the dark with occasional shaking. The mixture should not in some hours acquire a yellow or brown tint and no coloured precipitate should separate.

**Peroxides.**—The occurrence of these in commercial ether has at times misled analysts. The following tests seem to be the most satisfactory yet suggested.

*Vanadic Acid Test.*—0.1 gm. of vanadic acid and 2 c.c. of concentrated sulphuric acid are mixed in a porcelain dish, heated for about

15 minutes on the water-bath and cooled. This reagent, which should be red-brown, is dissolved in water, made up to 50 c.c., 1 or 2 c.c. of the yellowish liquid placed in a test-tube, 5 to 10 c.c. of the sample added, and the mixture well shaken. In the presence of peroxides, the mixture becomes rose to blood-red.

**Benzidine Test.**—A mixture of a saturated solution of benzidine and a 10% solution of copper sulphate gives with ether free from peroxides a brick-red, but in the presence of peroxides, a grayish green.

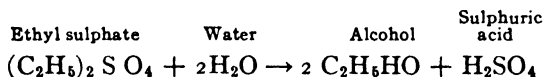
**Methyl-ethyl Ether.**—According to Frierichs (*Apoth. Ztg.* as above) the following method will serve to detect this possible contamination. 250 c.c. of the sample, of which the b. p. has been carefully determined, are distilled until 50 c.c. have passed over, using the same thermometer as employed in the original determination. If the b. p. is essentially lower, methyl-ethyl ether is present.

**Formaldehyde.**—The tests for this substance are given under that title in this section, but the following has been suggested for the special purpose of detecting it in ether and distinguishing it from acetaldehyde (*Jour. Soc. Chem. Ind.*, 1918, from *Pharm. Ztg.*, 1918, 68, 255. A small amount of guaiacol carbonate (0.02 grm.) is dissolved in 1 c.c. of the sample, 2 drops of ferric chloride solution added, and this mixture floated on the surface of some concentrated sulphuric acid. In the presence of formaldehyde, a cherry-red line will appear at the junction, but acetaldehyde does not give any colour.

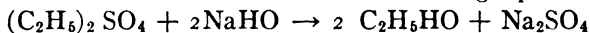
**Spirit of ether**, British Pharmacopœia, is a solution of about 28 parts of ether in 72 of rectified alcohol. The United States Pharmacopœia preparation is prepared by mixing 325 c.c. of official ether and 675 c.c. of official alcohol. *Compound spirit of ether* is made by substituting 25 c.c. of "ethereal oil" for an equal quantity of the alcohol in the simple spirit, otherwise as in that substance.

**Esters.**—These very numerous substances, often called compound ethers, are, structurally, the substitution of the ionisable hydrogen of acids by hydrocarbon radicles, but as a class they do not ionise appreciably in any solution, and, therefore, do not show the reactions of the acid radicles they contain. They are, however, subject to certain characteristic decompositions, by which the structural arrangement and composition may be determined. The principle types of these decompositions are *hydrolysis* and *saponification*.

*Hydrolysis* is the reaction of water with the ester by which the hydrocarbon radicle is converted into an alcohol, and the acid radicle into a free acid. The change may occur in small amount by mere contact of the ester with water, but is often greatly promoted by the action of a catalyst. A type of a hydrolytic reaction is



*Saponification* is brought about by the use of metallic hydroxides, especially those of sodium and potassium. The alcohol is formed as in the above reaction, and a salt of the acid with the metal of the hydroxide. Such salts were first obtained by treatment of the common fats and oils (which are esters of a special type, the chemistry of which is presented in another section) and were called *soaps*. A saponification reaction is shown in the following equation.



Very many esters have been prepared. Many of them have agreeable odours and are used as perfumes and flavours. The odours of many fruits and flowers are due to esters, and as these can be made artificially by comparatively simple processes, many such artificial products are now on the market.

Many esters have offensive odours and are very poisonous. Among these are compounds containing sulphur and phosphorus in an unoxidised condition, that is, not in the form of the radicles of the common acids derived from these elements. Several of them are highly toxic even when their vapours are diffused in high dilution in air. The preparation and properties of these substances have acquired great importance of late years on account of their extensive use in warfare. Recently, attention has been given to the possibility of using them for exterminating animal pests, such as rats and mice, and also as stenchers to be added to natural gas and fuel water-gas, which are without strong odour, and, therefore, liable to escape without detection (*Tech. Paper* 244, 1920, U. S. Bur. Mines).

Of the numerous gases that were employed in the late war, three deserve some special mention on account of their comparatively simple composition and highly irritating powers.

**Chlorethyl sulphide**,  $(C_2H_4Cl)_2S$ , mustard gas, often called dichlor-diethyl sulphide. It is a powerful blistering agent, and

acts quickly and severely on the eyes causing temporary blindness. It also produces hoarseness and inflammation of the lungs.

**Chloropicrin**,  $\text{CCl}_3\text{NO}_2$ , Trichloronitromethane.—The non-systematic name is due to the fact that the substance is produced by the action of bleaching powder on picric acid. It is liquid at ordinary temperatures.

**Carbon Oxychloride**,  $\text{COCl}_2$ , Phosgene.—This is a lethal gas of high activity.

For an account of the general methods of manufacturing and using these gases, see *J. I. E. C.*, 1919, **II**, 5. For a list of gases used in the war, see an article by Berolzheimer, in the same volume, p. 256.

The substance used in saponifying esters is usually dissolved in alcohol or glycerol. The latter liquid, suggested by Leffmann and Beam, is especially applicable to the saponification of the complex esters contained in fats, fixed oils and waxes, and its preparation and use is described in connection with the analysis of that class of substances. Aqueous solutions are very slow in action and the direct action of high-pressure steam is suitable only to operations on the large scale.

Many esters are hydrolysed at ordinary temperatures by catalysts. Such an agent is found in the secretion of the pancreas; another very active one in the castor bean.

The following are the details of the ordinary process of saponification, which is practically identical with that of Koettstorfer for the examination of fats:

A volume of 50 c.c. (measured with the greatest attainable accuracy) of a solution of potassium hydroxide (about 60 grm. in 1000 c.c. of alcohol) is introduced into a strong bottle holding about 100 c.c. A weighed quantity of the ester (from 4 to 6 grm.) is then added in such a manner as to avoid loss. This may be contained in a small glass bulb, or a known weight dissolved in pure alcohol may be added. The bottle is closed with an india-rubber stopper firmly secured by wire, heated to  $100^\circ$  for half an hour, allowed to cool, opened, a few drops of phenolphthalein solution added, and the liquid at once titrated with normal acid. A blank experiment is made by heating 50 c.c. of the reagent alone for half an hour and titrating. The *difference* between the acid required in the blank experiment, and that in which the ester was present, is acid corresponding to the

alkali neutralised by the ester. Each cubic centimetre of normal acid represents 0.0561 grm. of potassium hydroxide, or, in other words, each 1 c.c. of *difference* between the volume of the acid originally employed and that used in the blank experiment represents *one equivalent* in milligrams of the ester present.

As an example: Suppose that 45 c.c. of normal acid were employed in the blank experiment, and that 8 c.c. were required after saponification. The difference of 37 c.c. represents the alkali taken for the decomposition of the ester. As each centimetre of this contains 56.1 mg. or one equivalent in milligrams of alkali, it follows that the sample contained a number of milligrams equal to 37 times its equivalent. Supposing the weight of ester was 4.810 grm., then its equivalent would be  $4810/37 = 130$ . Of course, the equivalent thus found is identical with the molecular weight,  $1/2$  or  $1/3$  of the same, according to the constitution of the ester.

Conversely, if the equivalent of the ester is known to be 130, the weight of it present in the quantity of the sample taken will be  $130 \times 37 = 4.810$  grm.

This method often furnishes valuable evidence of the purity of the substances examined. An elementary analysis would scarcely detect 10% of ethyl alcohol in ethyl acetate, or of amyl alcohol in amyl acetate, but the saponification process would indicate these with certainty.

After decomposing the ester and titrating with acid, further knowledge may be obtained as follows:

The free alcohol is removed by distilling or evaporating the liquid after rendering it slightly alkaline. The residue is treated with an amount of sulphuric acid double that sufficient to neutralise the alkali originally added (*i. e.*, to produce potassium hydrogen sulphate), and the liquid is distilled. The acid of the esters will be liberated, and, if volatile without decomposition, will pass more or less into the distillate, and may be further examined. If such operation is to be applied, sulphuric acid should be used in the titration.

This method may be employed for the determination of chloroform and chloral hydrate when in alcoholic solutions.

Each c.c. of *difference* in the amounts of normal sulphuric acid required will represent 0.0299 grm. of chloroform or 0.0331 grm. of chloral hydrate.

For determination of esters in spirits, see page 257.

Many fruits owe their flavour to mixtures of esters, principally those containing the alcohol radicles of the methyl series. It has become a common practice to use artificial esters for imitating such flavours. The complete analysis of the mixtures sold is often impossible at present, but special ingredients may be detected. For important salicylic and benzoic esters, see vol. III, of this work.

The following examples of formulas for commercial artificial flavours appeared in a recent publication. The proportions are parts by volume added to 100 parts of well rectified alcohol (see p. 134).

**Pineapple Flavour.**—Chloroform, 1 part; aldehyde, 1 part; ethyl butyrate, 5 parts; amyl butyrate, 10 parts.

**Strawberry Flavour.**—Ethyl nitrite, acetate and formate, each 1 part; ethyl butyrate, 5 parts; amyl butyrate, 2 parts; amyl acetate, 5 parts.

**Raspberry Flavour.**—Ethyl nitrite, acetate, formate, butyrate, benzoate, cœnanthylate and sebacate; methyl salicylate; amyl acetate and butyrate, aldehyde: each 1 part.

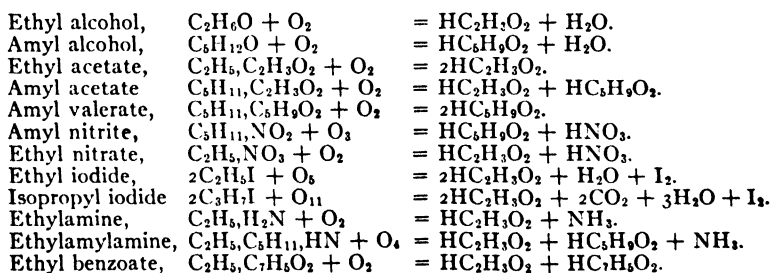
In the examination of these mixtures, chloroform and allied substances can be detected and estimated by conversion into hydrochloric acid, as described on page 344; aldehyde by the methods on page 332, nitrite can be detected even in minute amounts by Greiss' test (page 310) and, if present in notable quantity, can be estimated by the standard method of assay (page 313).

The fruit esters are usually saponified by the method given on page 307. As many of them are highly volatile, the operation must be conducted in a flask connected closely with a well-cooled return condenser. A freshly-prepared solution of pure sodium hydroxide in alcohol as free as possible from esters and aldehyde may be used. Small amounts of methyl alcohol may be expected, as methyl salicylate is often present. Alcohol is, of course, to be avoided as the solvent for the saponifying agent, when it is desired to detect or estimate ethyl as one of the radicles present. Water or glycerol may be substituted in such cases. The saponification with water is usually slow; a strong solution should be employed and the mixture heated for some time either in a strong, tightly-closed flask or under a return condenser. In all cases the condenser should be well cooled. The glycerol solution is made by mixing 20 c.c. of a 50% solution of sodium hydroxide with 180 c.c. of good glycerol.

The sodium salts resulting from the saponification may be separated by distilling the mass, care being taken not to overheat it, which would develop empyreumatic substances that interfere seriously with subsequent tests. The distillate contains the alcohols produced by the saponification, and the volatile, unsaponifiable ingredients, such as chloroform and aldehyde. By adding sulphuric or phosphoric acid in slight excess to the solid residue and again distilling, especially in a current of steam, some of the acids may be obtained in the distillate and may be recognised by odour and special tests. It is often impossible to distil all the acids present in this way.

A general method of examining esters was devised by Chapman and Smith (*Jour. Chem. Soc.*, 1871, 19, 477). It is based on the fact that organic substances when oxidised in a sealed tube by a mixture of sulphuric acid and potassium dichromate yield proximate products of oxidation closely related to the radicles contained in them.

The process consists in heating a known weight of the substance in a sealed tube for some hours with an aqueous solution, containing from 3 to 8% of potassium dichromate and 5 parts by weight of concentrated sulphuric acid to every 4 parts of dichromate. The following reactions were verified by the authors of the method as occurring almost in theoretical proportion.



Compounds containing methyl yield formic acid by oxidation, but the greater part of this is further oxidised to carbon dioxide and water. Chapman and Smith (*Jour. Chem. Soc.*, 1872, 20, 173) further showed that the process was capable of being used for investigating the structure of isomeric bodies. This is exemplified in the equation representing the oxidation of isopropyl iodide.

The assay of commercial esters may usually be conducted as described under *ethyl acetate*. A few, however, owing to their special properties or great individual importance, will be considered in separate sections.

**Ethyl Acetate.**—This is one of the most familiar esters, and enters into the formulas of many imitation fruit flavours. It occurs in many wines and in wine vinegar; and is produced spontaneously in many pharmaceutic preparations, notably in a solution of ferric acetate in alcohol. It possesses considerable solvent powers.

It is a colourless, fragrant liquid having a sp. gr. 0.893 to 0.898 at 25° and boils at 75°. It is miscible in all proportions with alcohol, ether and chloroform, but is only sparingly soluble in water, requiring 10 volumes at 25°. The solubility of water in ethyl acetate is 1 volume in 26 at 0°, and 1 in 24 at 15°. In a saturated solution of calcium chloride, ethyl acetate is but very slightly soluble, requiring 47 volumes at 15°, and almost as large a proportion at 0°.

Commercial ethyl acetate is often impure. In a series of eight samples representing the products of most of the leading makers, W. Inglis Clark found proportions of real ethyl acetate ranging from 90.14 to 30.6%; the alcohol from 7.2 to 48.0%; the free acetic acid from a trace up to 7.0%; while the other impurities (estimated by difference) ranged from 1.5 to 29.6%.

For the analysis of the commercial product the following process gives satisfactory results:

Dissolve 5 c.c. in proof spirit (freed from acid by adding a few drops of phenolphthalein, and then dropping in dilute alkali until a faint pink tint remains after shaking) and titrate with decinormal alkali. Each 1 c.c. neutralised represents 0.006 gm. of free acetic acid in the 5 c.c. used.

Add to another quantity of 5 c.c. of the sample the volume of alkali that has been employed in the titration, and then saponify the neutralised liquid as described on page 304. Each 1 c.c. of normal alkali neutralised by the sample represents 0.088 gm. of *ethyl acetate* in the quantity of the sample used, or 0.046 of alcohol regenerated from the ester.

20 c.c. of the sample are mixed with 20 c.c. of water and about 12 gm. of solid sodium hydroxide and placed in a flask with an

inverted condenser. After maceration for about an hour at room temperature, the mass is heated for 2 hours at  $100^{\circ}$ , then 20 c.c. of water are added and the mixture distilled until 50 c.c. are collected. The alcohol in the distillate is determined in the usual way. It should be tested for methyl alcohol (see p. 140). The weight so found is divided by 4 and from the quotient is subtracted the amount of alcohol produced from the ethyl acetate ascertained by the Köttstorfer method on page 304. The difference is the alcohol present as such in 5 c.c. of the sample.

By subtracting the sum of the *acetic acid*, *ethyl acetate* and *alcohol*, found as above, from the weight of 5 c.c. of the sample, the total amount of other impurities may be ascertained.

A very simple and approximately accurate method of ascertaining the proportion of real ethyl acetate present consists in agitating 10 c.c. of the sample, in a graduated tube, with an equal volume of a saturated solution of calcium chloride. The layer which rises to the surface is ethyl acetate. The results are fairly accurate, if the water and alcohol of the sample do not together much exceed 20% by volume, but with larger proportions the volume that separates is sometimes notably below the real amount of ethyl acetate present. The error may be avoided in some measure by adding to the sample twice its volume of ethyl acetate that has been previously treated with calcium chloride solution. 20 c.c. of the fortified sample should then be shaken with 20 c.c. of calcium chloride, when the diminution in the volume of the ethereal layer will represent the measure of impurities in  $20/3 = 6.67$  c.c. of the sample.

This method is due to W. Inglis Clark. The employment of water previously saturated with washed acetic ether, and coloured with a rosaniline salt, does not give satisfactory results.

The sp. gr. of ethyl acetate is not a satisfactory indication of its purity, as it dissolves alcohol, ether, and chloroform in all proportions and may be diluted with a spirit of approximately the same sp. gr. as the pure substance.

It should not contain more than a trace of free acid, and should be entirely volatile without residue.

25 c.c. shaken in a graduated tube with an equal volume of water previously saturated with pure ethyl acetate and the liquids allowed to separate, should give an upper (ether) layer measuring not less than 22.5 c.c.

A small portion of ethyl acetate poured upon strong sulphuric acid should not develop a dark ring at the point of contact of the two liquids.

**Nitrous Ethers.**—Two of these are of importance, owing to their use in medicine. Several other substances having formulas apparently identical with the derivatives of nitrous acid are, in reality, derivatives of nitric acid. Such are nitrobenzene and nitroethane. The true nitrous ethers (esters) are capable of saponification (see page 303) yielding a nitrite. Very minute amounts of nitrite can be detected by Griess' test, which consists in adding to the liquid to be tested a solution of sulphanilic acid, and then a solution of  $\alpha$ -naphthylamine, each dissolved in strong acetic acid. A nitrite will produce a red liquid. Only a very minute amount of the substance to be tested should be used and the solution should be allowed to stand for 5 minutes. It must be borne in mind, however, that nitrites are present in the air, water or even dust; hence error from these sources must be excluded. Commercial spirit of nitrous ether gives this reaction without saponification. One drop of the spirit in 50 c.c. of water is easily detected.

**Ethyl Nitrite.**—This ester has been known in an impure state for a long time. The method of preparation commonly used renders it liable to contain a number of impurities.

Pure ethyl nitrite is a nearly colourless liquid of fragrant odour. It is soluble in all proportions in alcohol, but requires about 50 parts of water for solution. It boils at  $18^{\circ}$ , and has a sp. gr. of 0.917 to 0.920 at  $0^{\circ}$ . It is liable to decompose on keeping, especially in presence of water. It gives many of the ordinary reactions of the nitrites. Thus, when mixed with a little dilute sulphuric acid, and poured on a strong aqueous solution of ferrous sulphate, it develops a dark-brown ring; when dissolved in alcohol and treated with a few drops of dilute sulphuric or acetic acid, it liberates iodine from potassium iodide, and therefore the mixture becomes blue on addition of starch.

The impure form has been known for a long time as "*sweet spirit of nitre*" (London Pharmacopœia, 1745). In addition to ethyl nitrite, water and alcohol, which may be regarded as normal ingredients of the commercial article, it may contain aldehyde, possibly paraldehyde, ethyl acetate and nitrate. Spontaneous changes occur on keeping, by which sensible amounts of acetic and nitrous acids are

developed. Additional impurities have been indicated, among which are: ethyl oxide, formate and oxalate, cyanides, glyoxal, glyoxalic, oxalic, malic and saccharic acids, and Allen suggested the possible presence of a substance isomeric with ethyl nitrat, enitroethane, which has a markedly different sp. gr. and b. p.

The tendency to gradual deterioration, with destruction of the nitrous ether, is a point of importance. The exact conditions are not thoroughly understood, but it is established that excess of water favours decomposition. Hence, adulteration with water, a common practice, not only dilutes the preparation, but greatly enhances the tendency to decomposition. Allen found that a sample kept perfectly well for many months, when undiluted, but a portion of the same mixed with one-third its volume of water contained no nitrous ether after an interval of 4 months. In these experiments the samples were kept in well-closed bottles. Imperfect closing of the bottle, exposure to light or to excessive temperature, will cause loss of nitrite. On the other hand, a solution of the pure ester in nearly absolute alcohol was kept 7 years and still contained ethyl nitrite and mere traces of free acid.

*Analysis of Spirit of Nitrous Ether.*—The assay of spirit of nitrous ether is somewhat difficult, on account of the complex character of the preparation. The sp. gr., behaviour with sodium acid carbonate, and reaction with ferrous sulphate in presence of free acid are serviceable. The U.S.P. requirements are a sp. gr. of 0.823 at 25°, and not less than 3.5% nor more than 4.5% of ethyl nitrite.

The following methods are fairly satisfactory.

*Excess of water* can be detected by the sp. gr. If it exceeds 0.853, excess of water is indicated. Commercial samples are sometimes adulterated so largely with water as to bring the sp. gr. to 0.910 or even higher. A sp. gr. of 0.845 corresponds, according to the accepted alcohol tables, with a content of 81.36% by weight of absolute alcohol, or 151.78% by volume of proof spirit. The extent to which a sample has been diluted with water may be found by multiplying the percentage of proof spirit (as found by the table) by the factor 657 ( $= \frac{100}{151.78} =$ ), when the product will be the percentage by volume of spirit of nitrous ether of standard contained in the sample. To find the percentage by measure of spirit of

0.850 sp. gr. originally present, the percentage of proof spirit in the sample should be multiplied by 0.673 (= 100/148.8).

Ethyl nitrite, though of higher sp. gr. than alcohol, is present in too small proportion to affect sensibly the determination of water from sp. gr.

*Free acid* will be indicated by the reaction with litmus paper, and the effervescence occasioned on adding sodium carbonate. A notable proportion of free acid renders the spirit incompatible with potassium iodide, from which it liberates iodine. The proportion of acid may be ascertained by titration with standard alkali, but, as some samples contain both free acetic and free nitrous acid, it is sometimes of interest to determine them separately, which is done by P. MacEwan in the following manner: 10 c.c. measure of the sample is placed in a flask containing a drop of phenolphthalein solution, and two or three drops of methyl orange solution are next added. A porcelain slab, spotted with drops of methyl orange solution, is arranged in readiness. N/2 solution of sodium hydroxide is then rapidly added to the contents of the flask, and as soon as the red begins to fade, a drop of the liquid is removed by means of a glass rod and brought in contact with a spot on the plate. If this assumes a pink tint, the nitrous acid is not quite neutralized, in which case the addition of the alkali solution is continued, until, on retesting, a spot is rendered only faintly pink. The volume of standard alkali used is then noted, and the titration continued until the pink tint produced by the phenolphthalein denotes alkalinity. Each c.c. of the alkali first used represents 0.0235 grm. of nitrous acid, while each c.c. of the additional alkali requisite to produce the phenolphthalein reaction corresponds to 0.0300 grm. of acetic acid. The process is approximate only.

When freshly prepared, or even after being kept for some time, spirit of nitrous ether should not be acid to litmus. Even when quite old it should not effervesce with potassium hydrogen carbonate.

*Aldehyde* will be indicated by the brown produced on heating the sample with potassium hydroxide. A sample free from an excessive proportion of aldehyde, when treated at the ordinary temperature with half its volume of a dilute solution of potassium hydroxide, assumes a yellow colour which gradually deepens, but does not become brown in 12 hours.

The United States Pharmacopœia test for presence of aldehyde is:

If 10 c.c. of the spirit are mixed with 10 c.c. of potassium hydroxide of 3% strength, the mixture will assume a yellow colour which should not turn decidedly brown within 12 hours.

*Ethyl chloride* and other *chlorinated substances* may be detected by igniting a little of the sample in a porcelain basin and holding a beaker moistened with silver nitrate solution over the flame. If silver chloride be formed, the sample may be evaporated with sodium hydroxide and the chloride in the residue determined.

*Total Ethyl Nitrite*.—The following is a summary of the United States Pharmacopœia process for ascertaining the amount of ethyl nitrite in spirit of nitrous ether.

Shake 30 grm. of the sample with 0.5 grm. of potassium hydrogen carbonate, transfer the liquid to a tared flask marked at 100 c.c., weigh accurately, add sufficient alcohol to make 100 c.c., and mix well. Put 10 c.c. of this mixture into a nitrometer, add 10 c.c. of potassium iodide solution (20%) and 100 c.c. of N/1 sulphuric acid. Allow the volume of evolved gas to become constant—which will require from 30 to 60 minutes—and note amount. Multiply this by 0.307 and divide by the weight of the sample taken. At the standard temperature (25°) and at the usual standard pressure (760 mm.) the quotient will be the percentage of ethyl nitrite in the liquid. To correct for temperature deduct  $\frac{1}{3}$  of 1% for each degree above the standard temperature, and add  $\frac{1}{3}$  of 1% for each degree below that temperature. To correct for pressure add  $\frac{4}{30}$  for each millimeter above the standard and deduct  $\frac{4}{30}$  for each millimeter below the standard.

The following special requirement is also given by the United States Pharmacopœia: if a test-tube is half-filled with the spirit and put into a water-bath heated to 65° until it has acquired this temperature, the liquid should boil distinctly upon the addition of a few small pieces of broken glass.

*Ethyl nitrite* may be detected by the brown coloration produced by adding ferrous sulphate to an acidified solution of the sample of spirit. Of various ways of making the test, Allen found the following the best: 10 c.c. of the spirit is mixed with 5 c.c. of a strong aqueous solution of ferrous sulphate. Pure, concentrated sulphuric acid should next be poured down the side of the test-tube in such a manner as to form a distinct stratum under the spirituous mixture.

A brown zone will thereupon be produced at the junction of the two layers, the intensity of which is an indication of the strength of the sample in nitrous ether. With good samples, the colouration is increased and extended by causing the layers to become partially mixed, but with inferior specimens the brown colour is more or less destroyed by such treatment (see also Griess' test, page 303).

*Methylated spirit* is said to be occasionally employed for the preparation of sweet spirit of nitre. For tests for other denaturants see page 138. The substitution may be detected by agitating 30 c.c. of the sample with 3 or 4 grm. of ignited potassium carbonate, treating 15 c.c. of the decanted dehydrated spirit in a small flask with 10 grm. of anhydrous calcium chloride, attaching a condenser, and heating the flask in boiling water till about 5 c.c. has passed over or scarcely any further distillate can be obtained. The operation proceeds slowly, but requires little attention and should be carried out thoroughly. The contents of the flask are next treated with 5 c.c. of water, and another 2 c.c. distilled. This second distillate is then oxidised by potassium dichromate and sulphuric acid, as described on page 307, and the product tested with silver nitrate. If the sample is free from methyl alcohol, the solution darkens, and often assumes transiently a purple tinge, but continues quite translucent; and the test-tube, after being rinsed out and filled with water, appears clean or nearly so. If the sample contains only 1% of methyl alcohol (= 10 to 20% of methylated spirit), the liquid turns first brown, then almost black and opaque, and a film of silver, which is brown by transmitted light, is deposited on the tube. When the sample is methylated to the extent of 3 or 4% the film is sufficiently thick to form a brilliant mirror. The observations should be made by daylight.

*Nitrates* may be detected and estimated by applying the phenol-disulphonic acid method (Vol. III). The liquid should be saponified (see page 297) with sodium (or potassium) hydroxide, made up to a convenient volume, and an aliquot part evaporated on the water-bath and treated with the reagent as directed Vol. III, above.

**Concentrated Spirit of Nitrous Ether.**—For the convenience of pharmacists, some manufacturers prepare a mixture of nitrous ether and alcohol of definite strength, so that by adding a given volume of alcohol, a liquid of official strength is obtained. A

Philadelphia manufacturer has devised a plan of furnishing this concentrated form in sealed tubes of amber glass. These are well-cooled, the point broken, the contents promptly mixed with a pint of alcohol, and a little more than a pint of U.S.P. spirit of nitrous ether obtained. The concentrated preparation usually contains about 16% of ethyl nitrite. Under ordinary circumstances, the assay of this will include estimation of the amount of ethyl nitrite and of the impurities to which that substance is liable. The simplest plan seems to be to add a measured volume of the sample to a known volume of pure alcohol and examine the liquid according to the methods already given for the commercial spirit.

W. A. Pearson (*Amer. J. Pharm.*, 1908, **80**, 101) devised a process for ascertaining the amount of alcohol in the concentrated preparation.

**Ethyl Chloride.**—This has been used both as a general and local anæsthetic. It is a colourless, mobile, very volatile liquid, with a slightly sweet taste and a pungent but rather fragrant odour. The sp. gr. is 0.8510 at 12°. The b. p. is 12.5° at ordinary pressure. It is slightly soluble in water, freely so in alcohol and ether. These solutions do not show the ordinary reactions for chlorine. Owing to its high volatility it is generally marketed in sealed tubes. It is best to keep these containers in a dark cool place. The following tests for the purity of a sample are generally satisfactory.

*Fixed or slightly volatile impurities* may be detected by evaporating a limited amount as described in connection with ether (page 296). No residue appreciable to sight or smell should be left.

*Alcohol and Aldehyde.*—10 c.c. of the sample should be agitated with 10 c.c. of water at a temperature of 10°. After standing to permit the liquids to separate, the ethyl chloride should be allowed to evaporate spontaneously from the open tube, then to the water should be added 3 drops of a moderately strong solution of potassium dichromate and 5 drops of dilute sulphuric acid. On boiling the mixture, no odour of aldehyde should be noted and the liquid should not assume a green or blue tint.

*Inorganic Chlorides, Especially Hydrochloric Acid.*—A 30 c.c. vial of ethyl chloride is attached to a glass tube containing 35 c.c. of water kept at 40°, and the chloride allowed to stream through the water until the container is empty. The water is then divided into several equal portions and these tested respectively with litmus paper,

which should not be reddened, with silver nitrate solution, which should give no turbidity, and the alcohol and aldehyde test may be applied to another portion.

**Ethylidene Chloride, Chlorinated Ethyl Chloride.**—This substance possesses anæsthetic properties, but its isomer, ethylene dichloride (Dutch liquid) produces convulsions when inhaled. Ethylidene chloride is also distinguished by not reacting with potassium, whereas Dutch liquid reacts violently, forming a porous mass, evolving hydrogen and chlorethylene,  $C_2H_3Cl$ , the latter being a gas of alliaceous odour. The same gas is produced when Dutch liquid is heated with alcoholic solution of potassium hydroxide, whilst ethylidene chloride is unaffected.

The b. p. of ethylidene chloride is between  $58^\circ$  and  $60^\circ$ ; that of Dutch liquid is between  $83^\circ$  and  $85^\circ$ . The sp. gr. of ethylidene chloride is 1.178 at  $15^\circ$ ; that of Dutch liquid is 1.265 at  $15^\circ$ . From chloroform, ethylidene chloride is distinguished by the b. p., sp. gr., and by not reacting with Hofmann's test (see page 344).

**Ethyl Bromide.** Hydrobromic Ether.—This has been employed in medicine as an anæsthetic. It boils at  $39^\circ$ , and has a sp. gr. of 1.455 at  $15^\circ$ . It burns with difficulty, giving a bright green but smokeless flame, and forming fumes of hydrobromic acid. The b. p. and smokeless flame distinguish it from ethyl chloride.

It is fairly stable when pure. It is liable to contain ethyl ether, which reduces the sp. gr. Some samples are contaminated with an acid impurity that has an extremely unpleasant odour, and is less volatile than ethyl bromide. For general tests see under Ethyl Chloride. The following tests are also available:

A small amount of the sample shaken in a clean bottle with concentrated sulphuric acid should not colour the acid after standing for 1 hour.

Shaken with water, the latter should not be increased in volume and should not acquire any acid reaction, nor give a precipitate with silver nitrate.

A few c.c. of the sample allowed to evaporate spontaneously should not develop any unusual odour.

A mixture of 1 c.c. of the sample with 3 drops of aniline and 2 c.c. of an alcoholic solution of potassium hydroxide should not give the isonitrile odour, thus indicating the absence of chloroform.

0.1 grm. added to 30 c.c. of 80% alcohol and 2 grm. of silver nitrate will give after several hours a precipitate of silver bromide, which should weigh 1.72 grm.

**Ethyl Iodide.**—This substance has been much used in organic research, especially in connection with the preparation of substitution ammoniums. It boils at from  $70^{\circ}$  to  $75^{\circ}$ , has a sp. gr. of 1.74 at  $15^{\circ}$ , is insoluble in water, but soluble in alcohol.

**Ethyl Carbamate.**—Carbamic acid is aminocarbonic acid. It forms colourless, odourless crystals having a sharp, somewhat cooling taste. It is soluble in less than its weight of water and alcohol, in about an equal weight of ether, in 1.3 parts of chloroform and in 3 parts of glycerol. These proportions are for a temperature of  $25^{\circ}$ . It melts between  $47.5^{\circ}$  and  $50^{\circ}$  and at a higher temperature it is decomposed, burning without leaving an appreciable residue.

The following tests for purity are condensed from the United States Pharmacopœia. Ethyl carbamate mixed with 5 times its weight of sulphuric acid and heated evolves carbon dioxide and leaves alcohol and ammonium hydrogen sulphate in the liquid.

Ethyl carbamate heated with concentrated potassium hydroxide solution yields ammonium hydroxide.

A solution of ethyl carbamate in water, mixed with sodium carbonate and a little iodine and warmed, will on cooling deposit iodoform.

Strong solution of ethyl carbamate in water should give no precipitate with nitric acid, mercuric nitrate or oxalic acid.

The assay of ethyl carbamate can be carried out by the ordinary Kjeldahl method. One hundred parts of the carbamate will yield 51.7 parts of alcohol and 19.1 parts of ammonia ( $\text{NH}_3$ ).

**Amyl Acetates.**—As eleven forms of amyl alcohol are possible, and the commercial forms may contain varying amounts of these, it is obvious that amyl acetate, which is derived from the alcohols, will differ considerably in different samples. The common product, however, consists largely of the isoamyl acetate, so that it is fairly uniform. It boils at  $148^{\circ}$  and has a sp. gr. of 0.8963 at  $0^{\circ}$ . Amyl acetate has a strong odour resembling that of the banana. It has considerable solvent powers and is used in some industries. It dissolves celluloid.

Amyl acetate may be estimated by the general method on page 304. From *alcohol* it may be separated by agitating the liquid with

an equal volume of saturated solution of calcium chloride which only dissolves the alcohol.

*Amyl alcohols* may be separated and estimated approximately by treating the sample in a graduated tube with a mixture of equal volumes of glacial acetic acid and water, which dissolves amyl alcohols, but not amyl acetate (together with any amyl valerate or pelargonate which may be present). By first separating the ethyl alcohol by salt water, or petroleum spirit, this method may be applied to the examination of the essence of *jargonelle pear*.

**Amyl Nitrites.**—The same conditions of isomerism apply to these compounds as to the acetates, but, similarly, the dominant form in the commercial product is isoamyl nitrite. The pharmacopœia requirements do not include a specification of the proportion of any particular form, but merely that 80% of nitrite should be present.

*Commercial amyl nitrite* is a yellowish liquid, with a penetrating, somewhat apple-like odour and a pungent, aromatic taste. Observations as to odour and taste should be made with care, as the concentrated substance, like most nitrites, has a powerfully poisonous action. Amyl nitrite is somewhat combustible, producing a smoky flame. It is almost insoluble in water, but freely miscible with methyl, ethyl and amyl alcohols, glacial acetic acid, ether, chloroform, carbon disulphide, benzene, petroleum spirit and oils. It is usually dispensed in small glass beads. In contact with air and probably influenced by light, it decomposes, becoming acid. Sp. gr. 0.865 to 0.875; b. p. 97° to 99°.

Concentrated sulphuric acid attacks amyl nitrite with great energy, red fumes being evolved, and a black, foul-smelling liquid formed. Occasionally the mixture inflames.

A characteristic test for amyl nitrite is the formation of potassium valerate when the liquid is dropped on melted potassium hydroxide. When gently warmed with excess of an aqueous solution of potassium hydroxide, potassium nitrite is formed, and a stratum of amyl alcohol floats on the surface of the liquid. The change occurs more readily by using the alcoholic solution and subsequently adding water to cause the separation of the amyl alcohol. On removing the aqueous liquid, acidifying it with acetic acid, and adding potassium iodide, the nitrite will occasion an abundant liberation of iodine. Griess' test can also be used (page 310).

Amyl nitrite, distilled slowly with methyl alcohol, is decomposed with formation of amyl alcohol and methyl nitrite. Ethyl alcohol causes less change, but it is evident that an alcoholic solution of amyl nitrite is liable to undergo decomposition.

The following qualitative tests are of use:

5 c.c. of the sample are shaken a few times with 10 c.c. of water, 1 c.c. of potassium hydroxide, and a drop of phenolphthalein solution. The colour of the liquid should not be entirely discharged.

A mixture of 1.5 c.c. of N/10 silver nitrate and 1.5 c.c. of alcohol with a few drops of ammonium hydroxide should not become brown or black if mixed with 1 c.c. of the nitrite and gently heated. This is the method suggested for detecting aldehyde which would reduce the silver salt.

A considerable number of impurities may be present in the commercial product, among which are ethyl and amyl alcohols, amyl nitrate, butyl and hexyl nitrites (derived from the presence of the corresponding alcohols in the fusel oil employed), nitropentane, valeric aldehyde, water and substances not specifically identified. If care is taken to employ amyl alcohol of definite quality in the preparation of the nitrite, most of these impurities will be present only in minute amount. The following table shows the nature, approximate sp. gr. and b. p. of the most important of such of these impurities as are not elsewhere noted.

	Sp. gr.	B. p.
Nitropentane.....	0.877	150-160
Amyl nitrite.....	0.870	98°
Amyl nitrate.....	1.000 at 7°	148
Amyl alcohol.....	0.814 at 15°	128-131
Valeraldehyde.....	0.8057 at 17°	92 5

Valeraldehyde is not likely to be removed by fractional distillation, though the other impurities can be more or less eliminated. Admixture of valeric aldehyde or amyl alcohol reduces the sp. gr. whilst amyl nitrate increases it. The latter has a comparatively high b.p.; hence, an instructive examination of amyl nitrite can be made by distilling the sample with a dephlegmator and noting the volumes, sp. gr. and odours of fractions collected at different temperatures. A fairly pure article, when fractionally distilled, will yield at least

80% of its original volume between 90° and 100°, and should leave no considerable residue at the latter temperature. Some specimens have been found to boil at temperatures ranging from 70° to 180°, and occasionally to leave a residue at 220°. As a rule, incomplete distillation at 100° is due chiefly to the presence of amyl alcohol, some of which may be formed by partial decomposition of the nitrite during distillation. Hence, commercial amyl nitrite of good quality may leave a residue of 5 to 10% at 100°.

A further examination of the nature of the 90° to 100° fraction might be made by gently heating it for some time with methyl alcohol in a flask furnished with an inverted condenser. On subsequent distillation, the fraction passing over between 90° and 100° will consist mainly of the *valeraldehyde* of the original sample, the amyl nitrite having been converted into amyl alcohol and the very volatile methyl nitrite.

*Nitropentane*, a body isomeric with amyl nitrite, appears to be present in most commercial specimens of the latter, and sometimes in notable quantity. It may be detected by subjecting the fraction distilling between 140° and 170° to the action of nascent hydrogen when amylamine will be formed, and may be recognised by the alkaline character of the distillate obtained on boiling with potassium hydroxide. Nitropentane may also be detected by dissolving the 140° to 170° fraction in solution of potassium hydroxide, adding a little sodium nitrite, and then dilute sulphuric acid very cautiously, when a blood-red tint will be produced, which disappears when the solution becomes acid. Pentyl nitrolic acid is produced and may be extracted by agitation with ether. Probably the test might be applied by warming the original sample with alcoholic potassium hydroxide and cautiously adding dilute sulphuric acid.

*Amyl nitrate*, if present, will be contained in the last fractions obtained on distilling a sample of amyl nitrite.

*Valeric aldehyde* may be detected by treating the sample with 3 volumes of a mixture in equal parts of strong ammonium hydroxide and absolute alcohol, then adding a few drops of silver nitrate solution and warming gently, when a dark-brown coloration will be produced if valeric aldehyde is present.

*Butyl and hexyl compounds* may be detected by saponifying the sample with sodium hydroxide and examining the amyl alcohol layer for butyl and hexyl alcohols by distillation.

*Free acid* may be detected and estimated as in spirit of nitrous ether after dissolving the sample in rectified spirit.

*Water* increases the sp. gr. of the preparation, and renders it turbid in melting ice. The presence of water increases the tendency to decomposition.

*Hydrogen cyanide*, occasionally present as a by-product, may be recognised by largely diluting the sample with alcohol and adding silver nitrate, when white curdy silver cyanide will be precipitated.

The United States Pharmacopœia process for the assay of amyl nitrite is the same as that for ethyl nitrite (page 311), except that 3 c.c. of the sample are shaken with potassium hydrogen carbonate and then decanted and weighed in the tared 100 c.c. flask. The reading of the volume of gas, multiplied by 4.8 and divided by the weight of sample taken gives the percentage of nitrite present at 25° and 760 mm. Corrections for differences of temperature and pressure are made according to the rule for assay of ethyl nitrite.

**Methyl anthranilate**, the methyl ester of ortho-aminobenzoic acid,  $C_6H_4(NH_2)(COOCH_3)$ , has of recent years been observed to be a widely distributed natural product. It was first found by Walbaum in neroli oil. The properties of the ester and the methods for its detection have been carefully investigated by F. B. Power, working in the Phytochemical Laboratory of the Bureau of Chemistry (*Amer. Chem. Soc.*, 1921, **43**, 377). His work was directed especially to the detection of the ester in fruit juices, especially grape juice. It was found that a preliminary extraction of the substance cannot be satisfactorily obtained by shaking with ether. The best method is to distil the juice in a current of steam. 500 c.c. may be employed and 200 c.c. of distillate collected. It is not necessary to neutralize the acidity of the original juice. If methyl anthranilate is present in any considerable amount the distillate has a blue fluorescence. The ester is extracted from the distillate by shaking with three successive portions (10 c.c. each) of chloroform. The mixed portions are passed through a dry filter and carefully evaporated in a small beaker over a water-bath in a current of dry air, the procedure being stopped as soon as the last portions of solvent are removed. The residue is at once treated with 2 c.c. of 10% sulphuric acid, and the solution transferred to a test-tube. The residue from the chloroform must not be exposed for an appreciable time to the air. The liquid in the test-tube is cooled, a drop of a 5% solution of sodium

nitrite added and then a few crystals of urea to destroy any nitrous acid. Two tests are recommended, the first being considered the more delicate.

*$\beta$ -naphthol Test.*—A solution of  $\beta$ -naphthol is prepared by dissolving 0.5 gram. of the pure substance in 2 c.c. of 10% sodium hydroxide and diluting to 100 c.c. A 10% solution of monohydrated sodium carbonate is also prepared. The test solution is made by mixing equal portions (1 c.c.) of the naphthol solution, a 10% solution of sodium hydroxide and the sodium carbonate solution. To this mixture, 2 c.c. of the solution to be tested are added, when even minute amounts of anthranilate will produce a yellowish red precipitate. The anthranilate is diazotized by the action of the nitrous acid.

*Dimethylanilin Test.*—Two c.c. of the diazotized liquid are mixed with 1 drop of dimethylanilin, and when the latter has dissolved, a slight excess of 10% sodium hydroxide is added. If not less than 0.001 gram. of the ester is present, a yellowish tint will appear, and on slightly acidifying the liquid with sulphuric acid, the tint will change to red, intensity of the colours thus produced being in proportion to the amount of the ester present.

The properties of methyl anthranilate are given by Power as follows: m.p.  $24.5^{\circ}$ ; b.p.  $127^{\circ}$  at 11 mm. It is readily soluble in dilute mineral acids, chloroform, alcohol, ether, light petroleum, and many other organic solvents, and to a slight extent in water. It is volatile with steam. Its solution generally shows a bluish fluorescence. This effect will probably be more evident if the liquid is examined by ultra-violet light. A piece of pine wood moistened with hydrochloric acid gives with the ester an intense orange colour.

## ALDEHYDES

The aldehydes are a series of compounds intermediate in composition between the alcohols and their corresponding acids. Many of them are liable to pass into polymeric forms or condensation products, especially when impure. Pure forms often remain unchanged for a long time.

Aldehydes are very readily oxidised to the corresponding acids. Hence, they are powerful reducing agents, precipitating metallic silver from the ammonio-nitrate and decolorising permanganates.

Nascent hydrogen (sodium amalgam) reduces aldehydes to the corresponding alcohols, but the fixation of hydrogen is often attended with condensation, and formation of a higher diatomic alcohol.

When heated with solutions of alkalis, they are mostly converted into resinous substances. By fusion with potassium hydroxide they are converted into the potassium salts of the corresponding acids, hydrogen being simultaneously evolved; in some cases this acts on another portion of the aldehyde and converts it into the corresponding alcohol. Many form compounds with water, hydrogen chloride and other substances, but the products are usually unstable.

Aldehydes readily combine with ammonia ( $\text{NH}_3$ ), the products first formed often undergoing molecular condensation. Ammonia compounds of the aldehydes of the acetic series are not liable to this change, and are stable crystalline substances which liberate the original aldehyde on treatment with dilute sulphuric acid.

Many aldehydes and allied substances (ketones) have the property of forming stable crystalline compounds with acid sulphites. The sodium compound is readily obtained on treating the aldehyde or its aqueous solution with excess of a saturated cold solution of sodium hydrogen sulphite, when the compound separates in crystals which are soluble in water or alcohol, but insoluble in a strong solution of the reagent.<sup>1</sup> From this the aldehyde may be regenerated by treatment with dilute sulphuric acid (or sodium carbonate), or sometimes by simply warming the aqueous solution. Aldehydes of the acetic series (as also chloral) reduce hot Fehling's solution, but aldehydes of the aromatic series do not.

Most substances of the aldehyde class give coloration with an acid solution of rosaniline previously mixed with sufficient sodium sulphite almost to decolourise it. (See page 326.) Examined in this way, acetaldehyde, paraldehyde, and propionaldehyde give an intense red-violet. Chloral gives at once a colour, but chloral hydrate, which is really not an aldehyde, gives no reaction. Acrolein and butyl chloral produce a violet coloration on shaking. Furfural and

<sup>1</sup> Neutral alkali-metal sulphites act upon many aldehydes and ketones with the separation of the hydroxide of the metal, thus:  $\text{R.HO} + \text{Na}_2\text{SO}_3 = \text{R.NaSO}_3 + \text{NaOH}$ . If an indicator such as phenolphthalein or rosolic acid is present, the change in reaction is indicated. With these indicators the, otherwise, practically colourless solution becomes red. In many cases the amount of the aldehyde, if its identity be known, can be estimated by titrating the hydroxide formed. This should be done in the presence of 50% glycerin and any original acidity should be neutralized by some of the standard acid prior to the addition of the neutral sodium or potassium sulphite. This process was first suggested by one of the American Editors. **AMER. EDS.**

benzaldehyde give the reaction more readily. Salicylic and cuminic aldehydes react well after some agitation. Cinnamic aldehyde and furfur-acrolein give at first an intense yellow, soon changing to violet red. Acetone readily reacts on shaking, but acetophenone and benzophenone have no action. Methyl and ethyl alcohols give a faint violet on shaking, propylic and isopropylic alcohols a scarcely perceptible reaction, whilst with their higher homologues, and phenols, glycols, quinine, sugars, and formic acid, no visible reaction occurs.

A mere trace of most substances of the aldehyde class produces a fine scarlet with a solution of phenol in excess of sulphuric acid, the colour changing to a dark red on warming the mixture.

A delicate test for aldehydes is the violet-red produced with diazobenzene-sulphonic acid in presence of free alkali. 1 part of freshly prepared diazobenzene-sulphonic acid is dissolved in 60 parts of cold water and rendered alkaline with sodium hydroxide. To this solution is added the liquid to be tested (previously mixed with dilute solution of sodium hydroxide) together with a little sodium amalgam. If an aldehyde is present, an intense violet-red is produced, either immediately or within 20 minutes. The colour is destroyed by long exposure to the air, and is changed by the addition of an acid. Diazobenzene-sulphonic acid is produced by adding a small amount of sodium nitrite to a dilute solution of sulphanilic acid containing some acetic acid.

The reaction is readily yielded by a solution containing 1 part in 3000 of benzaldehyde, and has been obtained with acetic, valeric, and cœnanthic aldehydes, as also with furfural and glyoxal. Chloral and benzoin do not give the reaction. Acetone and ethyl acetoacetate give a red coloration, but without the violet tint characteristic of an aldehyde. The reaction is not produced by phenol, resorcinol, or pyrocatechol (if care be taken to have excess of alkali present), but is given by dextrose. It is said to be more delicate than that with rosanilin reduced with sulphurous acid; but is more especially suitable for the detection of aldehydes which are permanent in alkaline solutions.

The ammoniated silver solution described on page 333 is a general test for aldehydes.

**Acrolein, valeral, furfural** and certain constituents in the **essential oils** of bitter almonds, cinnamon, cloves, cumin, and meadow-sweet

have the constitution and characters of aldehydes. All these form crystalline compounds with acid sulphites.

**Acetone and acetal** are substances allied to the aldehydes, and **chloral** is a trichloraldehyde.

According to Ripper (*Monatsch. d. Chem.*, 1900, **21**, 1079) any aldehyde soluble in water or in very dilute alcohol can be assayed as follows: (See page 334.)

A convenient amount of the solution of the aldehyde is mixed with twice its volume of a solution of acid potassium sulphite (containing 12 grm. in 1000 c.c.). The mixture is allowed to stand for 15 minutes, and the unprecipitated sulphite determined by titration with iodine. The sulphite solution must, of course, be valued by a similar titration.

**Formic Aldehyde. Formaldehyde.**—Methaldehyde.

This substance is produced by the limited oxidation of methyl alcohol. It presents a general resemblance to ordinary or acetic aldehyde, but it is polymerised with extreme readiness. It is gaseous at the ordinary temperature. A polymer, paraformaldehyde, is a white insoluble substance, subliming at the temperature of boiling water, and suffering depolymerisation at a higher temperature, or when heated to  $140^{\circ}$  with excess of water in a sealed tube.

Ordinary formaldehyde undergoes slow oxidation in the air, to formic acid. It is rapidly oxidised by the more powerful oxidising agents. When its aqueous solution is mixed with solid potassium permanganate, a violent reaction occurs, some of the aldehyde is oxidised to carbon dioxide and water, and another portion escapes in the form of vapour. The gases eliminated are often combustible. This reaction has been utilised for obtaining formaldehyde vapour in disinfecting large inclosed spaces.

Formaldehyde reacts with ammonium hydroxide forming a substitution amine (see page 331). When heated with sodium hydroxide for some time on the water-bath, formaldehyde forms sodium formate and methyl alcohol.

The solid polymer is now sold under the name "paraform" for disinfecting purposes. This material begins to sublime at  $100^{\circ}$  and melts between  $153^{\circ}$  and  $172^{\circ}$ , producing gaseous formaldehyde.

The United States Pharmacopœia solution of formaldehyde (*Liquor formaldehydi*) is required to contain not less than 37% by weight of the aldehyde. The sp. gr. of this solution ranges from

1.075 to 1.081 at 25°. It should not contain more than 0.2% of free acid, calculated as formic.

Formaldehyde has acquired great importance within the last few years on account of its employment as a disinfectant and food preservative! The literature concerning it is extensive; much of it relates to the detection of the substance in food, especially milk. It is principally sold as a 40% (by volume) solution in water, under the name "formalin." Formaldehyde forms compounds with many substances, often rendering them highly insoluble.

When solutions of formaldehyde are boiled, a considerable portion passes over with the steam, but if the distillate be transferred to a dish on the steam-bath and evaporated, much of the substance will remain as a white solid—the polymeric modification.

Many tests for formaldehyde have been published. Some of these are general tests for the aldehydes (see page 322). The following are mostly of this character, but they are especially employed for the detection of formaldehyde.

*Fuchsin Test.*—This is performed with Schiff's reagent, for the preparation of which Allen suggested the following: 40 c.c. of a 5% solution of magenta (fuchsin) are mixed with 250 c.c. of water, 10 c.c. of sodium acid sulphite solution of 1.375 sp. gr., and then 10 c.c. of pure sulphuric acid. The mixture is allowed to stand for some time, when it will become colourless. The addition of a solution of formaldehyde restores the red of the dye, but a colour resembling that caused by formaldehyde may be obtained by blowing air through the reagent, by contact with aerated water or even by warming.

*Betanaphthol Test (Mulliken, Ident. Pure Org. Comps., vol. 1).*—The solution to be tested is mixed with 3 c.c. of dilute alcohol (1:2), 0.005 grm. of betanaphthol and 3 to 5 drops of hydrochloric acid and boiled for a few minutes. Any precipitate is collected on a filter, washed with dilute alcohol of the same strength as that first used, dissolved in a small amount of alcohol by the aid of heat, the liquid cooled, the crystals that separate collected on a filter, washed with 1 c.c. of strong alcohol, dried on a porous tile in a warm place and the m. p. determined. The precipitate when formaldehyde is present is methylene-dibetanaphthol, melting at 189°.

<sup>1</sup>As far as the United States is concerned, the vigilance of the Pure Food Authorities has practically driven formaldehyde out of any use in foods. AMER. ED.

*Phenylhydrazine-nitroprusside Test.* Rimini's test—A small amount of phenylhydrazine hydrochloride is added to the solution to be tested, then a drop of dilute solution of sodium nitroprusside and a few drops of sodium hydroxide solution. The liquid becomes deep blue if formaldehyde is present. The nitroprusside solution should be freshly prepared. With milk containing formaldehyde this test produces a greyish-green.

*Phloroglucinol Test.*—A small amount of a freshly prepared (about 1%) solution of phloroglucinol in water is mixed with an equal volume of a 25% solution of sodium hydroxide, and the solution to be tested added. The liquid becomes rose-red. It is best to introduce the liquid to be tested by means of a pipette, so as to underlie the reagent solution. The colour then appears at the junction of the liquids.

*Bonnet's Test* (*J. Am. Chem. Soc.*, 1905, **27**, 601).—A small amount of morphine is placed on a watch glass, a drop or two of sulphuric acid added, the mass stirred with a glass rod, and the watch-glass floated on the surface of the liquid to be tested. The whole is then covered with a glass or porcelain cover and allowed to remain for at least 30 minutes. If formaldehyde is present the mixture in the dish will become dark. This test has the advantage that the reaction can only be due to a volatile ingredient, and the interfering or misleading reactions of substances in complex organic mixtures, such as milk, are avoided.

A. O. Gettler (*J. Biol. Chem.*, 1920, **42**, 311) who has subjected the tests for formaldehyde to a careful comparative examination, states that apomorphine is a more delicate reagent than morphine in this test. Only a few milligrams of the alkaloid should be used.

*Resorcinol Test* (Lebbin).—A few c.c. of the liquid to be tested are boiled with 0.05 gm. of resorcinol, to which half or an equal volume of a 50% solution of sodium hydroxide is added. If formaldehyde is present, the yellow solution changes to a fine red. Analogous compounds showing the usual reactions characteristic of aldehydes fail to give this colouration.

*Milk Test* (Hehner).—Milk containing formaldehyde produces with strong sulphuric acid a purple-violet liquid. The test is best applied by underlaying the milk with the acid, when the colour is seen just below the line of junction. The acid must contain a trace of iron, which can be easily secured by adding a drop or two of ferric

chloride to 5 c.c. of the pure acid. Good results are obtained by putting a few crystals of potassium sulphate into the milk before underlaying it with the acid.

*Crystallisation Test.*—Gettler uses the reaction with ammonia, which results in the formation of a tetramine, as a means of detection of formaldehyde. He gives the following directions:

10 c.c. are mixed with an equal volume of strong ammonium hydroxide solution in an evaporating dish, and brought to dryness on the water-bath. The crystalline residue is dissolved in a few drops of water, and a drop of solution of mercuric chloride added. Typical crystals of hexamethylene-amine mercuric chloride are formed. It is well to compare the crystals with those obtained from a solution of known character and purity. It is probable that the modern methods of studying the optical properties of crystals will be of value in identifying such precipitates but these methods require elaborate apparatus and special experience.

C. H. LaWall (*Proc. Pa. Pharm. Assn.*, 1905, 200) found that vanillin simulates formaldehyde in the sulphuric acid contact test and the resorcinol test. As vanillin is often used in association with foods that are likely to be preserved with formaldehyde, care must be taken not to overlook this simulation. LaWall found that the phenylhydrazine test is not given by vanillin.

#### QUANTITATIVE METHODS

The tests for formaldehyde are best applied to pure solutions in water. As the substance is readily volatile with steam, some of it can usually be obtained in satisfactory form by simple distillation, but the amount that passes over is uncertain, and it is often impossible to obtain a distillate containing all the formaldehyde. The estimation of the small quantities employed for preserving milk is especially attended with great difficulty. The preliminary isolation of the preservative by distilling the milk is open to objection, but the experiments made by Leonard and Smith (*Analyst*, 1897, 22, 5) show that rough indications of the amount of formaldehyde present can be obtained with certain precautions: (1) The distillate from fresh milk exerts no appreciable reducing action on alkaline permanganate, but milk 3 or 4 days old yields a distillate having marked reducing properties. (2) The separation of formaldehyde from milk is

facilitated by acidifying the liquid with sulphuric acid and blowing live steam through it. Under these conditions the first 20 c.c. of distillate from 100 c.c. of milk will contain about one-third and the first 40 c.c. about one-half of the total amount of formaldehyde present. (3) The fact that the distillate from milk does not contain the whole of the formaldehyde present is to a great extent explained by the behaviour of solutions of formaldehyde on distillation, and is only partly due to any specific action of the preservative on the constituents of milk.

Several methods of assay have been suggested, some of which are described below. According to R. H. Williams (*J. Am. Chem. Soc.*, 1905, **27**, 596) the iodine method is best adapted to pure dilute solutions; the potassium cyanide method to impure dilute solutions; the hydrogen peroxide method, directed by the United States Pharmacopœia, to strong impure solutions.

*Iodometric Method.* Romijn's method (*Zeit. anal. Chem.*, 1897, **36**, 18).—10 c.c. of the solution to be tested are diluted to 1000 c.c. with water and of this 10 c.c. are mixed with 25 c.c. of N/10 iodine and sodium hydroxide solution added, drop by drop, until the liquid becomes clear yellow. After 10 minutes hydrochloric acid is added and the free iodine is titrated with N/10 sodium thio-sulphate. Two atoms of iodine are equivalent to 1 molecule of formaldehyde. This method is suitable for the accurate determination of formaldehyde alone, but does not give good results in the presence of other aldehydes and ketones.

*Potassium Cyanide Method.*—This is based upon the fact that formaldehyde combines with potassium cyanide. The addition product reduces silver nitrate in the cold, but if the silver nitrate be acidified with nitric acid before the addition of the aldehyde mixture, no precipitate results if the aldehyde in the latter is in excess. If, on the other hand, the cyanide is in excess, 1 molecule of potassium cyanide is left in combination with 1 molecule of the formaldehyde, while the excess precipitates silver cyanide from the silver nitrate solution.

10 c.c. of N/10 silver nitrate, acidified with nitric acid, are mixed with 10 c.c. of potassium-cyanide solution (prepared by dissolving 3.1 grm. of the 96% salt in 500 c.c. of water), the whole diluted to 500 c.c., filtered, and 25 c.c. of the filtrate titrated by Volhard's method. The difference between this blank result and that obtained by titrating the filtrate after the addition of the aldehyde solution

gives the amount of N/10 sulphocyanate corresponding to the silver not precipitated by the excess of potassium cyanide. From this the amount of formaldehyde can be calculated. Results by this method are said to be correct, even in the presence of acetaldehyde, if the liquid is titrated immediately after shaking.

*Hydrogen Peroxide Method.*—This method, originally proposed by Blank and Finkenbeiner (*Ber.*, 1893, 31, 2979) has been subjected to much investigation and amendment, but the following form devised by Haywood and Smith (*J. Am. Chem. Soc.*, 1905, 27, 1185) has given good results and is more convenient than the older form. 50 c.c. of normal sodium hydroxide solution are measured into an Erlenmeyer flask of 500 c.c. capacity, and 50 c.c. of pure 3% hydrogen dioxide solution added. The liquids are mixed, and, by means of pipette, 3 c.c. of the formaldehyde solution added, allowing the tip of the pipette to come very near to the surface of the mixture. A funnel is then put in the mouth of the flask and the liquid heated for 5 minutes on the steam-bath, shaking occasionally. The flask is removed from the bath, the funnel washed down with water, the liquid allowed to cool down to room temperature, and the excess of sodium hydroxide titrated with standard acid, litmus being used as indicator. The sp. gr. of the formaldehyde solution being taken, the amount of actual formaldehyde present can be calculated. The formaldehyde is converted into formic acid.

The United States Pharmacopœia directs that the sample shall be weighed and gives as factor for calculation the following: the number of c.c. of alkali consumed by the formic acid produced by the oxidation, multiplied by 2.979 and divided by the weight of solution taken, will give the percentage by weight of absolute formaldehyde present.

Commercial solutions of formaldehyde and hydrogen peroxide are usually distinctly acid; hence, such acidity must be determined and allowed for in the calculation, or the liquids exactly neutralised with dilute sodium hydroxide, using litmus as an indicator, before beginning the analysis. Neutral and alkaline solutions of hydrogen peroxide are liable to decompose rapidly.

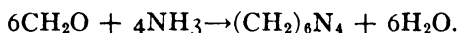
*Ammonia Method.*—A. G. Craig (*J. Am. Chem. Soc.*, 1901, 23, 642) finds that the reaction between ammonia and formaldehyde (see page 331) can be used according to the following manipulation:

Several stout bottles holding about 100 c.c. and provided with good rubber stoppers are selected, and a vessel used that will allow them to

be submerged while standing upright. In each of the bottles is placed 25 c.c. of normal ammonium hydroxide (it is not necessary that this should be exactly of that strength). In some of the bottles accurately measured volumes of the solution to be tested are placed, using a quantity containing about 0.5 grm. of the aldehyde.

The bottles are securely corked, tied down, placed in the heating vessel, submerged in cold water and the latter raised to the b. p. Water must be added cautiously from time to time, keeping the bottles submerged and upright. After being in the bath for 1 hour, they are removed, cooled, opened, a little methyl orange added to each and then the contents of each titrated with normal sulphuric acid until the *first red* tint appears. The difference between the amount of acid required for the blanks and that for the bottles in which the formaldehyde solution was placed is the equivalent of the ammonia used by the latter. 1 c.c. of normal ammonium hydroxide is taken up by 0.0601 of formaldehyde.

**Hexamethylene-tetramine. Hexamethylene-amine.**—This substance is a substitution amine (tetramine), but as it is a direct product from formaldehyde and owes its importance entirely to its relation to that substance, it will be described here. It is produced by the action of formaldehyde on ammonia, the equation reaction being



The compound forms colourless, odourless, glistening crystals, soluble in about 1.5 parts of water, both cold and boiling, 10 parts of alcohol, and sparingly soluble in ether. The solution in water is alkaline to litmus; tannin and mercuric chloride give precipitates with it. The solid is volatilised and partially decomposed by heating. Strong sulphuric acid converts it into ammonium sulphate and formaldehyde. A mixture of it with a little salicylic acid becomes red on warming with sulphuric acid.

It is apparently partly hydrolysed in the animal system, reproducing formaldehyde and ammonium hydroxide. The former is excreted with the urine. It is sold under many proprietary names, among which are "urotropin," "formin," "cystogen."

In addition to the tests above given, an assay of a sample could readily be made by the Kjeldahl-Gunning method. The pure substance contains 40 per cent. of nitrogen.

The frequent use of hexamethylene-amine in proprietary medicines and nostrums renders it necessary to note some processes especially adapted to detecting it in such articles. Horton found that bromine forms a brick-red precipitate, having the composition  $C_6H_{12}N_4Br_4$ , which on drying becomes yellow and is converted into the dibromide,  $C_6H_{12}N_4Br_2$ . Dobriner found that mercuric chloride produces precipitates which differ with the ratio of the reagent to the hexamethylene-amine; when the latter is in excess, the precipitate consists of monoclinic prisms containing  $2C_6H_{12}N_4 + 3HgCl_2$ , but when the reagent is in excess the compound  $C_6H_{12}N_4 + 6HgCl_2$  is formed.

The bromine and mercuric chloride compounds may be further tested for identification. The dibromide melts slightly below  $200^\circ$ . The amounts of bromine and nitrogen present may be ascertained by the usual methods. In the case of the mercuric chloride compound, which should be obtained by using excess of the reagent, the proportions of mercury and chlorine will be important data.

*Separation of Formaldehyde and Acetaldehyde.*—Mulliken and Scudder have described the following method:

A round-bottom flask is connected with a long spiral reverse condenser, through which water at from  $45^\circ$  to  $50^\circ$  is passed. The upper part of the spiral is connected tightly with a tube which turns and is connected with a descending spiral condenser which is surrounded with ice and salt and is tightly connected with a flask, also immersed in a freezing mixture. All the condensing apparatus should be of glass. A suitable amount of the aldehyde mixture is placed in the distilling flask and gently boiled for 2 hours. Only traces of formaldehyde pass into the receiver, but the acetaldehyde distils over. The liquid to be distilled should not contain over 1% of formaldehyde.

For other processes for distinguishing between formaldehyde and acetaldehyde.

**Acetaldehyde. Acetic Aldehyde. Ethyl Aldehyde.**—This is the substance from which the class of aldehydes derived its name, and which is indicated when the term “aldehyde” is used without qualification.

It is a colourless, mobile liquid, with a pungent, suffocating odour. The disagreeable odour is much stronger in the crude substance. Its sp. gr. is 0.790 and it boils at  $22^\circ$ . It does not redden litmus,

but on exposure to air, oxidises slightly to acetic acid. It is miscible in all proportions with water, alcohol, and ether. It is insoluble in a saturated solution of calcium chloride, but this property is not available for the quantitative separation of it from alcohol. A practicable method is to treat the liquid with dry calcium chloride, which forms a compound with the alcohol, when the aldehyde may be distilled off by the heat of a water-bath.

When kept in closed vessels, aldehyde often passes into liquid or solid polymers, especially in presence of traces of mineral acid. An alcoholic solution is tolerably permanent. Phosphoric anhydride and concentrated sulphuric acid, when heated with aldehyde, turn it thick and black, but it may be distilled from sulphuric acid diluted with an equal weight of water.

Aldehyde is a powerful reducing agent. It separates metallic silver from the ammonio-nitrate, when gently warmed, an acetate being formed in the liquid. The reaction is rendered more delicate by the addition of alkali. A suitable test solution may be prepared by mixing equal volumes of 10% aqueous solutions of silver nitrate and sodium hydroxide, and then adding ammonia, drop by drop, till the oxide of silver is dissolved. The reagent should be freshly prepared, as it is liable to decompose with deposition of fulminating silver. It yields an immediate mirror with a liquid containing 1% of aldehyde, and in half a minute with a solution containing 1 in 1000, whilst 1 part of aldehyde in 10,000 of water yields a yellow-brown mirror in 5 minutes. The solution should be previously distilled, as several common organic substances slowly reduce the reagent. Acetaldehyde also gives a precipitate of cuprous oxide when heated with Fehling's solution.

Strong solutions of caustic alkalies produce with aldehyde a reddish-brown mass, only slightly soluble in water and known as "aldehyde-resin." On adding, for instance, sodium hydroxide to a rather strong solution of aldehyde a yellowish-red precipitate appears, a disagreeable gas is evolved and the reddish-brown resin separates. The reaction constitutes a qualitative test for acetaldehyde, and has been also utilised by Thresh (*Pharm. Jour.*, [3], 1878-9, 9, 409) as a means of estimating it, for which purpose he recommends the following procedure:

The solution to be tested should be diluted so that it contains not more than about 1 part of the aldehyde in 200 parts of water.

To 200 c.c. of such solution, 30 c.c. of a sirupy solution of sodium hydroxide should be added, and the mixture kept at the b. p. for a few seconds. It is then cooled, diluted with 200 c.c. of alcohol and made up to 500 c.c. by addition of water. A mixture of methyl and ethyl alcohol may be employed provided it is free from all but traces of acetaldehyde. The solution should be clear and light-yellow. To determine the aldehyde, the solution should be compared with a dilute solution of potassium dichromate, which has been standardised by means of aldehyde solutions of known strength, as in the ordinary colour-comparisons, such as Nessler's test.

*Rocques' Method of Estimating Aldehyde* (*J. Phar. Chim.*, [6], 1898, 8, 390, 497).—An alcoholic sulphite solution is prepared by dissolving 12.6 gram. of sodium sulphite in 400 c.c. of water, adding 100 c.c. of N/1 sulphuric acid, and making up to 1000 c.c. with 95% alcohol of the highest purity. The solution is allowed to stand overnight and filtered from the sodium sulphate.

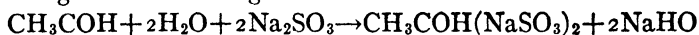
A suitable volume of the sample, which should not contain more than 2% of aldehyde, is placed in a flask marked at 100 c.c., an accurately measured volume of the alcoholic sulphite solution is added, and the liquid is made up to 100 c.c. with 50% alcohol of highest purity. The flask should have a long neck and be well closed after the mixture is made up. A similar flask is prepared with the diluted reagent alone. The flasks are kept at 50° for 4 hours, cooled, and the contents titrated with standard iodine (with starch) in the usual manner. Rocques uses different strengths of iodine according to the amount of aldehyde present, but for ordinary cases he prescribes N/1 solution. If the sulphite solution has been properly made, it and the iodine solution will be equivalent volume for volume. 1 c.c. of N/1 iodine equals 0.0032 gram. of sulphur dioxide or 0.0022 gram. of acetic aldehyde.

For a method of preparing a standard aldehyde solution and for further information in reference to the estimation of aldehyde, see p. 259.

Aldehyde also combines with ammonia ( $\text{NH}_3$ ) forming a crystalline substance of the formula  $\text{C}_2\text{H}_4\text{O.NH}_3$ , or  $\text{CH}_3\text{CH}(\text{NH}_2).\text{OH}$  (amido-ethyl alcohol), insoluble in ether and decomposed on distillation with moderately dilute sulphuric acid.

For a special process for separating formaldehyde and acetaldehyde, see pp. 332.

When a strong solution of acetaldehyde is mixed with a strong solution of neutral sodium sulphite, a violent effervescence occurs, but the evolved gases do not give indication of the presence of sulphur dioxide. The liquid becomes hot, a yellow precipitate forms which finally turns dark red, and, if the solution is very strong in aldehyde, the mass sets to a resinous solid. S. S. Sadtler has investigated this reaction and finds that it is dependent on the formation of an aldehyde sulphite compound and liberation of sodium hydroxide according to the following reaction:



A strong solution of formaldehyde does not show any visible phenomena under the conditions as noted above, but the reaction is presumably the same. The solid mass produced from acetaldehyde is probably the aldehyde resin resulting from the action of the sodium hydroxide.

Sadtler has founded a process for estimating aldehydes generally upon this reaction, having found it of general occurrence with both aliphatic and cyclic aldehydes.

*Betanaphthol Test.*<sup>8</sup>—About 0.25 gram. betanaphthol, 2 drops hydrochloric acid and 20 c.c. of glacial acetic acid are shaken until the naphthol is all dissolved. A drop of the solution to be tested is then added and the liquid heated to between 50° and 60° for 5 minutes, boiled for 1 minute, cooled and shaken vigorously until a precipitate settles. As the liquid tends to show the phenomenon of supersaturation, it will be well to allow considerable time and to stir actively with a glass rod before deciding that the result is negative. If a precipitate appears, it should be collected on a filter previously moistened with glacial acetic acid, washed with 1 c.c. of the same, then boiled with a mixture of 3 c.c. alcohol and 1 c.c. of water for half a minute. Much of the precipitate may not dissolve. The solution is cooled thoroughly, shaken actively, and the precipitate that separates collected on a filter, washed with 1 c.c. cold dilute alcohol (1 to 1) and dried for 30 minutes at 100°. If acetaldehyde was present in the liquid tested, the product is ethylidene-dibetanaphthol oxide,  $(\text{C}_{10}\text{H}_8)_2\text{O}(\text{C}_2\text{H}_4)$ , which melts at 172.5° to 173.5°.

**Paraldehyde,  $\text{C}_6\text{H}_{12}\text{O}_3$ .**—This polymeride is produced by adding a minute quantity of hydrochloric or sulphurous acid to ordinary aldehyde. On adding a drop of concentrated sulphuric acid to alde-

hyde violent ebullition occurs, much aldehyde is volatilised, and the residue consists of paraldehyde. Zinc chloride acts similarly, but calcium chloride and potassium acetate do not. The paraldehyde may be purified from unchanged aldehyde by cooling the liquid below  $0^{\circ}$ , when the crystals which separate are pressed between folds of blotting-paper and distilled.

Paraldehyde is a colourless, transparent liquid with strong odour and sharp taste. Its sp. gr. is 0.990 at  $25^{\circ}$  (United States Pharmacopœia). It is soluble in 8 parts of cold and 16.5 parts of boiling water; on account of the lower solubility at the higher temperature, the cold saturated solution becomes turbid on boiling. Paraldehyde is miscible in all proportions with alcohol and ether. It solidifies at about  $0^{\circ}$ , melts at  $10.5^{\circ}$  and boils at between  $121^{\circ}$  and  $125^{\circ}$ . The vapour is inflammable. The liquid is nominally neutral, but may be slightly acid to litmus. It possesses reducing power similar to ordinary aldehyde. The United States Pharmacopœia requires that 1 c.c. of paraldehyde shall form with 10 c.c. of water a clear solution, which must not form a precipitate with silver nitrate or barium chloride.

Paraldehyde does not show any noticeable reaction with sodium sulphite as described in connection with aldehyde (p. 335), but after standing for some time the mixture acquires a slight reddish tint, due possibly to a small amount of aldehyde in the commercial preparation.

**Metaldehyde**,  $\alpha\text{C}_2\text{H}_4\text{O}$ , is another polymeride produced simultaneously with paraldehyde (see above). It is insoluble in water, and almost insoluble in alcohol or ether, but dissolves somewhat in acetaldehyde. Its best solvents are hot chloroform and benzene. At ordinary temperatures the crystals are permanent in the air. It is reconverted more or less completely into ordinary aldehyde by repeated distillation or by heating in a sealed tube to  $110^{\circ}$  or  $115^{\circ}$  and readily by distillation with a little dilute sulphuric acid. Permanganates, chromic acid mixture, and ammonium hydroxide are without effect on metaldehyde, but chlorine at once converts it into ordinary chloral. With a hot strong solution of alkali, metaldehyde very slowly yields aldehyde-resin the reaction being probably preceded by formation of aldehyde.

*Determination of Acetaldehyde in Paraldehyde* (*Apoth. Zeit.*, 1913, 28, 720).—25 grm. of the sample are mixed with cooled water in a

wide-mouth flask until solution is complete, 30 c.c. of N/1 potassium hydroxide and 20 c.c. of hydrogen peroxide solution are added, the mixture cautiously shaken and allowed to stand over night. The excess of alkali is titrated, using phenolphthalein. The calculation is as follows:

$$\text{acetaldehyde} = \frac{\text{c.c. of unused solution} \times 0.04403 \times 100.}{25 \times \text{sp. gr. of sample}}$$

*Detection and estimation of acetone, acetaldehyde and formaldehyde in presence of each other.* The following procedures, devised by E. Pittarelli, originally described in Italian literature, are given from an abstract (*Chem. Zentralbl.*, 1920, **61**, 616).

If to a solution containing all of the above substances, hydrazin hydrochloride is added, then methyl orange, hydrochloric acid and mercuric iodide, a carmine-red precipitate forms in a few minutes if formaldehyde is present. This reaction is not given by the other two substances. Carbazol in alcoholic solution gives with formaldehyde, in the presence of strong sulphuric acid, a green colouration not given by the others. The aldehydes react with neutral hydrazin salts with the liberation of free acids while acetone gives this reaction only with hydroxylamin salts. This difference can be utilized as a quantitative reaction for the aldehydes in presence of acetone.

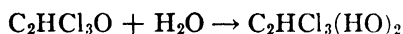
Neutral colourless solutions of rosolic acid in sodium sulphite are coloured violet by the aldehydes and acetone, by liberation of free alkali. The reaction is most sensitive with formaldehyde, least with acetone. Acetone and acetaldehyde give with hypobromites and phenols colour reactions not given by formaldehyde. They can also be identified by the isonitrile reaction, or by reduction to acetylene after conversion by bromine into bromoform. Acetone is better determined by the hydroxylamin method (titration of the mineral acid liberated from a hydroxylamin salt) than by the iodoform process.

In order to detect acetone in a mixture of formaldehyde and acetaldehyde two test samples are provided, some methyl-orange added, and the liquids exactly neutralized. To one is added a solution of phenylhydrazin, neutral to methyl orange, and to the other a similarly neutral solution of hydroxylamin. The indicator changes by reason of the liberation of free acid in the liquids. By titrating back to neutrality the difference of acidity between the phenyl-

hydrazin mixture and the hydroxylamin mixture, the amount of acetone can be determined.

**Acetal**,  $C_6H_{14}O_2$ , has the constitution of a di-oxyethyl-acetaldehyde:  $CH_3.CH(OC_2H_5)_2$ . It is produced by the action of aldehyde on alcohol, and hence is a constituent of crude spirit and of the "feints" obtained in the rectification of alcohol. When pure, acetal is a liquid of pleasant taste and odour, boiling at about  $105^\circ$  and having a sp. gr. of 0.821 at  $22^\circ$ . By oxidising agents it is converted into acetic acid and aldehyde, and when heated with acetic acid, it yields ethyl acetate and aldehyde. A dilute aqueous solution treated with sodium hydroxide and iodine forms a clear colourless liquid, which yields a dense precipitate of iodoform when acidified. From alcohol, acetal may be separated by distillation over dry calcium chloride, and from aldehyde and ethyl acetate by heating with strong solution of potassium hydroxide.

**Chloral. Trichloraldehyde.**—Chloral is not in itself a commercial article. It is prepared as a source of other substances. It is a colourless oily liquid of a sp. gr. of 1.544 at  $0^\circ$  and b. p. about  $96^\circ$ . It distils unchanged. Pure chloral is but little liable to change, but the ordinary substance is easily polymerized, producing *meta-chloral*, which is far less soluble in common solvents than the chloral itself. Alkalies quickly convert chloral into chloroform and a formate. The principal use of chloral is the formation of compound by reaction with water in accordance with the equation



which is really a trichlorinated glycol, but is erroneously called *chloral hydrate*, a name which is probably too firmly fixed in medical and pharmaceutic nomenclature to be avoided.

When chloral is mixed with an equivalent quantity of absolute alcohol it is converted into a substance called *chloral alcoholate*, which forms colourless crystals that melt at  $46^\circ$ , boil at  $113.5^\circ$ , and are freely soluble in chloroform. These properties, among others, serve to distinguish it from chloral hydrate.

**Chloral Hydrate.**—This substance, the name of which, as has been stated, is incorrect, is a colourless crystalline mass with characteristic odour. It is soluble in water (1 to 1.5 by weight), alcohol, ether, benzene, petroleum spirit, and carbon disulphide. When crystallised from the last solution it boils at  $97.5^\circ$  and melts at  $57^\circ$ .

When mixed with an equal weight of camphor or phenol it rapidly liquefies. The liquid has the mixed odour of its constituents and does not precipitate silver nitrate.

Chloral hydrate is soluble with difficulty in cold chloroform, requiring four times its weight; a fact which distinguishes it from the alcoholate, which is readily soluble. The alcoholate represents less chloral than the hydrate. Both compounds should be entirely volatilised on heating, and their solution in water should be neutral to ordinary indicators.

Solution of chloral hydrate gives no reaction with silver nitrate in the cold, but on boiling and adding a little ammonium hydroxide a mirror is readily produced. If kept some time, chloral hydrate contains a trace of hydrochloric acid, and the solution in water then gives a cloud with silver nitrate, but the production of a distinct precipitate indicates serious impurity.

When water is present chloral hydrate is deliquescent, and in warm weather even melts. Hence it is often made with a slight deficiency of water. If more than a shade short of this the product has a tendency to become acid, and ultimately partially insoluble from formation of metachloral.

In the following table are given other useful distinctions between chloral alcoholate and chloral hydrate:

	Chloral alcoholate	Chloral hydrate
1. M. p. ....	0.46°	57°-58°
2. B. p. ....	113.5°	97.5°
3. Sp. gr. of the fused substance at 66°:	1.344	1.57
4. Sp. gr. of the aqueous solution at 15.5°:		
5% .....	1.007	1.019
10% .....	1.028	1.040
15% .....	1.050	1.062
20% .....	1.071	1.085
5. Gently heated with nitric acid of 1.2 sp. gr.	Violently attacked.	Scarcely acted on.
6. Shaken with an equal volume of strong sulphuric acid.	Brown.	No visible change.
7. Warmed with 2 volumes of water.	Melts without complete solution, and on cooling congeals below the surface.	Readily dissolved.
8. Heated on platinum foil .....	Inflames readily.	Scarcely burns.
9. With alkali and iodine .....	Gives iodoform.	Gives no iodoform.

The solidifying point of melted chloral hydrate is a datum of some value. The sample should be placed in a small test-tube, fused, and the tube immersed in water at about  $55^{\circ}$ . A thermometer is placed in the liquid, and the temperature at which it becomes opalescent noted. The best quality solidifies at about  $48^{\circ}$  to  $49^{\circ}$ , and the best *practically* adjusted specimens within half a degree of  $50^{\circ}$ . A low freezing point indicates excess of water, and such specimens are liable to deliquesce. Small granular crystals and saccharoid masses are purer than large crystals or needles.

The b. p. is also of service as a test of purity. The sample should be placed in a test-tube with some broken glass. A pure sample will begin to boil rapidly at  $97^{\circ}$ , and the temperature will change but little until one-half has been volatilised. The material, however, undergoes slow decomposition at the b. p., so that the first portions of the distillate are under-hydrated, and the last over-hydrated. The b. p. consequently undergoes a gradual rise. The best commercial specimens, *i. e.*, those slightly under-hydrated, begin to boil throughout the liquid at about  $96.5^{\circ}$ . The under-hydrated portion boils off in a few seconds, and the b. p. rises to  $97^{\circ}$ , and finally to  $97.5^{\circ}$  or  $98^{\circ}$ , by the time half has boiled away. A b. p. above  $98^{\circ}$  indicates an over-hydrated and deliquescent sample. If the boiling fairly commences below  $95^{\circ}$ , the sample is too much under-hydrated, and is liable to decompose on keeping.

**DETECTION AND DETERMINATION OF CHLORAL.**—These procedures have acquired considerable importance of recent years on account of the not infrequent employment of the drug in alcoholic solution, as the so-called “knock-out drops,” for drugging liquor to facilitate the commission of robbery or rape.

Chloral hydrate may be detected by the same means as chloroform (page 344). It reduces Fehling’s solution on heating. The reaction may be employed to detect traces of chloral if other reducing substances are absent, and might probably be made quantitative.

Traces of chloral may be detected by Hofmann’s test for chloroform (see page 344); also, by boiling the liquid and passing the vapour through a red-hot tube, when hydrochloric acid will be formed, and the condensed water will precipitate silver nitrate.

For the estimation of real *chloral*, advantage may be taken of the reaction with alkalis, which results in the separation of chloroform and the production of a formate.

K. Müller gives the following method: 25 gm. of the sample are placed in a finely graduated tube, and a strong solution of potassium hydroxide added, in quantity rather more than sufficient for the above reaction. A large excess must be avoided. The tube must be kept well cooled, as the action is very violent at first. Afterwards, the tube may be closed and the mixture shaken. After resting an hour or two the liquid becomes clear and separates into two layers. The lower layer is chloroform, and, after being brought to a temperature of  $17^{\circ}$ , the volume may be read off. Its sp. gr. may be taken as 1.49 hence the measure in c.c., multiplied by 1.84, gives the weight in gm. of anhydrous chloral in the quantity employed. If the factor 2.064 be substituted, the product will be the weight of chloral hydrate present. Müller obtained by this process an average of 71.6% of chloroform from pure chloral hydrate, against 72.3% as required by theory.

H. C. Wood proposed the following: 10 gm. of the sample are dissolved in 50 c.c. of water contained in a small flask, and 4 gm. of slaked lime are added. A cork with a tube bent twice at right angles is adapted to the flask, the outer end of the tube being somewhat drawn out and immersed in a small quantity of water, contained in a narrow graduated glass tube surrounded with cold water. A gentle heat is applied to the flask, and the chloroform slowly distilled over. After a few minutes the heat is gradually increased, so as to keep the mixture boiling, the operation being continued till 10 c.c. have passed over. Nothing remains but to bring the chloroform to the proper temperature and read off the volume. The addition of a few drops of potassium hydroxide solution destroys the meniscus of the chloroform, and enables the operator to observe the measure accurately. The process is brief. Too much lime occasions frothing, but an excess appears to have no decomposing action on the chloroform. Lieben's iodoform test for alcohol is readily applied to the aqueous portion of the distillate. Allen found this plan convenient and fairly accurate. A correction may advantageously be made for the slight solubility of chloroform. This is about 0.3 c.c. for every 100 c.c. of aqueous liquid.

A modification of the process has been suggested by Meyer. It has the advantage of being applicable to small amounts of material. 2 gm. of the sample are dissolved in water, and free acid removed by shaking the liquid with barium carbonate and filtering. The

filtrate is treated with a moderate excess of normal sodium hydroxide, and titrated back with acid in the usual way, litmus being used as an indicator. Each c.c. of normal alkali neutralised by the sample corresponds to 0.1475 grm. of chloral ( $C_2HCl_3O$ ), or 0.1655 grm. of chloral hydrate.

**Butyric Chloral. Butyl Chloral.**—Butyric trichloraldehyde. This is erroneously called croton chloral. It bears the same relation to butyl alcohol and butyric acid that ordinary chloral bears to ethyl alcohol and acetic acid.

Butyl chloral was at first called croton chloral because the hydrogen was underestimated, which led to the supposition that it was the trichlorinated aldehyde of crotonic acid, the fourth member of the acrylic or oleic acid series. It is a dense, oily liquid of peculiar odour, boiling at about  $163^\circ$ . When treated with a considerable excess of warm water it dissolves, and on cooling is deposited:

**Butyric Chloral Hydrate.**—This substance forms white, silvery crystalline scales melting at  $78^\circ$  and having a sweetish melon flavour. The sp. gr. is 1.695. Butyric chloral hydrate is but little soluble in cold water, but more so in hot. Its solubility is increased by addition of glycerol. It is very soluble in alcohol and ether, but insoluble, or nearly so, in chloroform. This last property may be employed to separate it approximately from ordinary chloral hydrate. It differs also from the latter substance in its m. p. and b. p. The two substances may also be separated by distillation, ordinary chloral hydrate passing over a little below  $100^\circ$ , while butyric chloral hydrate is decomposed into water, which distils at about  $100^\circ$ , and anhydrous butyric chloral boiling at about  $163^\circ$ . When acted on by alkalies, butyric chloral hydrate is at first decomposed with production of a formate and propylic chloroform, but this again splits up with formation of a metallic chloride and allylene dichloride.

**Allylene dichloride** is very unstable, being gradually decomposed even at ordinary temperatures, and acquiring an acid reaction and disagreeable odour. The proneness to change, so marked in some samples of commercial chloroform, and the readiness with which the latter decomposes and becomes acid, are properties possibly due to the presence of allylene dichloride. Its presence may be due to the existence of aldehyde in the crude alcohol used for the preparation of the chloroform. By the action of chlorine the aldehyde is converted into butyl chloral, and this by subsequent contact

with the calcium carbonate used for neutralisation, gives allylene dichloride.

**Chloralformamide.**—This is produced by the action of formamide ( $\text{CH}_3\text{NO}$ ) on chloral. It forms colourless, odourless crystals, soluble in about 19 parts of water at  $25^\circ$ . The solution is somewhat bitter and is neutral to litmus. One part of chloralformamide dissolves in 1.3 parts of alcohol, and it is also readily soluble in ether, glycerol, ethyl acetate, and acetone. Heated alone, it melts at about  $115^\circ$  and at a higher temperature volatilises leaving no appreciable residue. Its solution in water is not affected by acids, but is decomposed by sodium hydroxide with formation of chloroform. The solution in alcohol does not redden litmus nor produce a precipitate with a solution of silver nitrate.

**Chloroform. Trichlormethane.**—Chloroform is a colourless, heavy liquid of marked, but not disagreeable, odour, and sharp, sweetish taste. It is very volatile. The liquid is not combustible, and vaporises only with difficulty, but mixed with alcohol vapour it burns with a smoky flame edged with green. Very carefully purified chloroform was found by Baskerville and Hamor to have the sp. gr. of 1.49887 at  $15^\circ/4^\circ$ , and a b. p. from  $60^\circ$  to  $61^\circ$ . It is soluble in about 200 parts of cold water, and is miscible in all proportions with absolute alcohol, ether, benzene and petroleum spirit, and to a limited extent with dilute alcohol. It has a high range of solvent power, dissolving many alkaloids, fats, waxes, resins, camphor, rubber, gutta percha, iodine, bromine and phosphorus.

Several grades of chloroform are common in the market. That which is intended for industrial purposes is not as a rule required to conform to such strict standards as that intended for anæsthesia. Baskerville and Hamor<sup>1</sup> have considered in their investigations the properties and tests of pure and anæsthetic chloroform, but these tests will also serve for the examination of industrial products.

Pure chloroform is liable to spontaneous decomposition, especially when exposed to light and air. Acrid and irritating, volatile substances are produced, among which is usually carbon oxychloride. Such decomposition is prevented to a great extent by the presence of a small amount of alcohol, and, accordingly, chloroform intended for medicinal purposes is allowed to contain from 0.5% to 1.0% of that substance.

<sup>1</sup> J. Ind. Chem. Eng., 1911, 3, 301.

The sp. gr. of anæsthetic chloroform ranges from about 1.4730 to 1.4827 at 25°.

Chloroform has some antiseptic properties. A few drops added to 100 c.c. of urine will preserve the sample for months, and check any decomposition already in progress. The addition renders the copper reduction tests valueless, but does not interfere with the bismuth or phenylhydrazine tests. Sediments are also preserved, but only a small amount of chloroform should be added, as an excess will accumulate at the bottom of the container and interfere with the microscopic examination.

Chloroform can be freed from water and alcohol by the same processes recommended for purifying ether (page 300). The reaction with calcium carbide may also be of use.

When the quantity of alcohol exceeds 1 or 2%, the proportion may be ascertained with tolerable accuracy by shaking 20 c.c. of the sample in a graduated tube with 80 c.c. of water. If the chloroform is pure it will collect at the bottom in clear globules, but in the presence of alcohol the liquid and the surface of the drops will become dim and opalescent. The reduction in the volume of the chloroform shows the proportion of alcohol in the amount taken. The addition of a few drops of potassium hydroxide solution destroys the meniscus and enables the volume to be read more accurately. The aqueous liquid may be tested for sulphuric acid by barium chloride, for free chlorine or hypochlorous acid by starch and potassium iodide; for hydrochloric acid by silver nitrate; and the presence of alcohol definitely proved by the iodoform test.

### DETECTION AND ESTIMATION OF CHLOROFORM

A delicate method for the detection of chloroform in the presence of large quantities of alcohol has been described by A. W. Hofmann. All that is necessary is to add some alcoholic sodium hydroxide and a little aniline to the liquid to be tested. Either immediately or on gently warming the mixture, a strong and peculiar smell will be observed, due to the formation of phenyl carbamide (phenyl isocyanide). Bromoform and iodoform give the same reaction, as also do chloral, trichloroacetic acid, and all other substances which yield either of the above products by treatment with alkalis, but ethylidene chloride,  $C_2H_4Cl_2$ , gives no such product under these conditions. The test will show 1 part of chloroform dissolved in 5000 parts of alcohol.

Reduction of Fehling's solution is also a test for chloroform. When the solution is heated, the cuprous oxide separates promptly. Chloroethylidene and alcohol do not interfere with the test.

When chloroform vapour mixed with hydrogen is passed through a red-hot tube, it is decomposed with production of hydrochloric acid. This reaction is used for the detection and estimation of chloroform. The sample should be boiled in a small flask through which a current of hydrogen is allowed to pass. The mixed hydrogen and chloroform vapour are then caused to traverse a short length of heated combustion tube containing platinum wire-gauze or loose asbestos.

The products are passed through a bulb-tube containing water, and the hydrochloric acid is titrated with standard alkali, or precipitated with silver nitrate. 109.5 parts of hydrochloric acid, or 430.5 of silver chloride represent 119.5 of chloroform. Berthelot points out that the reaction with silver is apt to be vitiated by the presence of acetylene and hydrocyanic acid, and recommends that the aqueous solution of the gases should be well boiled before adding silver nitrate.

This process is especially useful for the estimation of small quantities of chloroform contained in other non-chlorinated liquids. It may be employed for the detection and estimation of chloroform in blood. When its detection only is required, a current of air may be substituted for the hydrogen. There is no occasion to heat the blood.

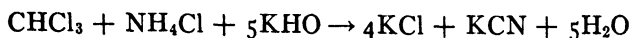
Vitali suggests that the mixture of hydrogen with chloroform vapour obtained as in the last reaction should be submitted to Hofmann's isonitrile reaction or passed through a freshly prepared mixture of thymol and solid potassium hydroxide, when if chloroform is present the mixture will be coloured a fine reddish-violet.

When chloroform is added to a solution of  $\alpha$ - or  $\beta$ -naphthol in strong potassium hydroxide, and the liquid is heated to about  $50^{\circ}$ , a fine blue is developed, changing in contact with the air to blue-green, green, green-brown and finally brown.

*Cyanide Test.*—Autenrieth (*Detection of Poisons*, 5th. Amer. ed., 37) recommends the following procedure:

A convenient portion of the sample is heated in a sealed vessel with a mixture of a little solid ammonium chloride and an alcoholic solution of potassium hydroxide. The heating should be for several

hours in boiling water. Dr. Warren, translator of the work, suggests the use of the familiar magnesium citrate bottle. After closing the bottle, a towel should be wrapped around it and the bottle then placed in cold water, which should be gradually raised to the b. p. After several hours have elapsed the liquid should be allowed to cool. The bottle should not be opened until everything is quite cold. The liquid should be tested for cyanide by the standard tests. The reaction is as follows:



A positive reaction for cyanides is proof that the sample contained chloroform. The possibility of explosion should not be forgotten, and the operation should be conducted in such a way as to avoid danger to person or property.

**Examination of Commercial Chloroform.**—The following tests are compiled from the reports of an elaborate examination made by Baskerville and Hamor.<sup>1</sup> The investigation applied especially to pure and medicinal samples, but the tests are also applicable to industrial products.

*Specific Gravity.*—The sp. gr. of pure chloroform should be taken by means of the pycnometer. For the figures see p. 343.

*Odour.*—100 c.c. of the sample should be slowly evaporated over a water-bath until 10 c.c. remain. This residue should have no unusual odour, and when allowed to evaporate at room temperature no odours should be observed except those of chloroform and ethyl alcohol.

*Fixed Residue.*—No weighable residue should remain when 100 c.c. are allowed to evaporate at 100°. The presence of residue should be tested by actual weighing and not by inspection.

*Organic Impurities.*—If 20 c.c. are mixed with 15 c.c. of concentrated sulphuric acid in a glass-stoppered tube of 50 c.c. capacity, which has been previously rinsed with some strong sulphuric acid, no visible colouration should be imparted to the liquid after adding 0.4 c.c. of 40% formaldehyde solution and shaking well for 5 minutes.

*Water.*—Pure chloroform and anhydrous anæsthetic chloroform must comply with the following test: when 20 c.c. of the sample are boiled over 1 gram. of clean crystals of calcium carbide, and the vapours passed into ammonio-silver nitrate, no evidence of the presence of acetylene should be given.

10 c.c. of anæsthetic chloroform agitated with an equal volume of paraffin oil (sp. gr. 0.880) should produce a clear solution.

*Alcohol*.—Determination of sp. gr. is important in this connection. For chemical tests the following are satisfactory:

10 c.c. are shaken in a separatory funnel with 4 c.c. of concentrated sulphuric acid; the extraction is repeated with another similar portion and then with 2 c.c. The sulphuric solution is mixed with 40 c.c. of water and then gently distilled until about 20 c.c. have passed over. To 10 c.c. of the distillate, 6 drops of 10% solution of potassium hydroxide are added and the liquid warmed to about 50°. A solution of potassium iodide fully saturated with free iodine is added, drop by drop, with constant shaking, until a permanent yellowish-brown colour results, when potassium hydroxide is added cautiously until the liquid is decolorised. No iodoform should be deposited. This test, as is well known, does not prove the presence of alcohol, but absolutely pure chloroform will conform to it. As the presence a certain amount of alcohol in anæsthetic chloroform is not objectionable, the sp. gr. determination will be of value, but some idea of the amount of alcohol in such samples can be gleaned by noting the amount of the deposit. For exact determination, see below (page 349).

*Acetone*.—A negative result with the iodoform test shows absence of acetone. For a direct test, the following should be used: 10 c.c. of the sample are shaken with 5 drops of a 5% solution of sodium nitroprusside and 2 c.c. of ammonium hydroxide (sp. gr. 0.925), and the mixture allowed to stand for a few minutes. In the presence of alcohol in moderate amount a yellowish-brown tint to the supernatant liquid may appear, but acetone produces an amethystine tint. The liquid must not be warmed. For further determination, 100 c.c. of the sample should be distilled, and the first 10 c.c. of distillate and the last 10 c.c. left in the flask should be tested by the method. If the proportion of acetone is about 1 to 500, the amethystine colour is marked, but in proportion of 1 to 1000, the colour may not appear until the liquid is saturated with ammonium sulphate, shaken and allowed to stand for 5 minutes. As acetaldehyde is not often present in fresh and properly stored samples of chloroform in proportion greater than 1 to 3300, usually the reaction is not disturbed by the presence of it, but in every case the examination for acetaldehyde should be made.

*Acetaldehyde*.—Chloroform of all grades should pass this test. 5 c.c. of the sample are agitated with 5 c.c. of François' reagent (see below) in a glass-stoppered tube. No colouration should appear after 15 minutes. Extremely small amounts of acetaldehyde can be detected by this method. Pure chloroform giving a negative result with the iodoform reaction may be regarded as free from acetaldehyde, but it should in addition give no response when 5 c.c. are shaken with an equal volume of Nessler's reagent, after standing 5 minutes. 10 c.c. of anæsthetic chloroform shaken with 10 c.c. of water and 5 drops of Nessler's reagent should not give any precipitate in 5 minutes, and no distinct colour should be produced, although a slight turbidity may appear.

*Acidity*.—20 c.c. are shaken with 10 c.c. of water and 2 drops of phenolphthalein solution, and then titrated with N/100 potassium hydroxide solution added drop by drop. Not more than 0.2 c.c. of this should be required to produce a faint but distinct alkaline condition permanent for 15 minutes when the mixture has been shaken for 30 seconds between each addition of alkali.

*Carbon Oxychloride*.—This highly objectionable impurity is best detected by the following test: To 15 c.c. of the sample in a dry glass-stoppered tube of 25 c.c. capacity, sufficient perfectly clear barium hydroxide solution is added to fill the tube, which is then allowed to stand for 3 hours in a dark place without shaking. No white film should form at the junction of the liquids.

*Hydrochloric Acid and Chlorides*.—Samples complying with the requirements of the test for acidity are assuredly free from these and carbon oxychloride, but pure and anæsthetic chloroform should also conform to the following requirement: When 10 c.c. are shaken with 5 c.c. of water for 5 minutes no precipitate should appear on adding silver nitrate, and no reduction to metallic silver should occur on warming.

*Free Chlorine and Hydrogen Peroxide*.—When 10 c.c. of the sample are shaken with 10 c.c. of a 10% solution of potassium cadmium iodide, no liberation of iodine should occur, as indicated by the addition of a little starch solution.

*Decomposition Products of Chloroform*.—Anæsthetic chloroform failing to meet with the test for acidity, and which contains none of the substances indicated in the tests just described, contains acetic acid and should be rejected.

When 20 c.c. of anæsthetic chloroform are shaken during 20 minutes with 50 c.c. of sulphuric acid in a glass-stoppered tube of 50 c.c. capacity, previously rinsed with sulphuric acid, and 2 c.c. of the acid are then diluted with 5 c.c. of water, the liquid should remain colourless and clear, no unusual odours should be noted except those due to chloroform and alcohol, and the liquid should retain its clear, colourless condition when further diluted with 10 c.c. of water and no turbidity appear on the addition of 5 drops of silver nitrate solution. A positive result in this test indicates the presence of chlorinated derivatives of the oxidation products of alcohol.

*Determination of Alcohol.*—The sp. gr. has been regarded by many as a satisfactory method, but while it is true that alcohol does materially modify the sp. gr. of chloroform, the effect is not in exact proportion to the amount present. Some observers have endeavored to overcome this defect by determinations of sp. gr. of known mixtures of the two substances, but such tables will be applicable only to samples containing only these two ingredients, a condition not likely to occur in practice.

Nicloux (*Bull. Soc. Chim.*, [3], 1906, **35**, 330) devised a process which is endorsed by Baskerville and Hamor, who, however, call attention to a modification by Behal and François (*J. Pharm. Chim.*, [6], 1897, **5**, 417).

In this modification, the sample is agitated with sulphuric acid, which removes the alcohol. The acid solution is then diluted with water and gently distilled. A portion of the distillate is boiled with sulphuric acid and potassium dichromate solution (16.97 grm. per 1000 c.c.) added until a green, but not a greenish-yellow, tint is reached. Each 2 c.c. of solution corresponds to 0.01 grm. of absolute alcohol. If the result should indicate a proportion of alcohol above 2 c.c. per 1000 of the sample, the distillate should be diluted with water and the process repeated.

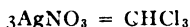
#### METHOD OF DETERMINING CHLOROFORM IN MEDICINAL PREPARATIONS

This method has been repeatedly used in the laboratory of the American Editors with satisfaction.

Weigh out 50 c.c. of the preparation in a glass-stoppered 50 c.c. graduated flask and transfer to a distilling flask, rinsing out the graduated flask three times with water so that the total solution will amount to not more than 100 c.c.

Distil slowly over a very small flame until two thirds of the liquid has passed over into 50 c.c. alcoholic potassium hydroxide. The receiving end of the condenser is connected up with an adapter, the end of which is under the surface of the liquid in the receiver. This precaution is taken so that none of the vapors of chloroform may escape. The alcoholic potassium hydroxide solution is placed in a pressure flask which acts as the receiver. After the distillation is finished (about 2 hours), the distilling flask is disconnected, the condenser rinsed with a small amount of water into the pressure flask. The latter is then stoppered and placed vertically in a water bath which is heated to boiling for 3 hours. Care should be taken that none of the liquid in the flask comes in contact with the stopper. After the proper length of time the flask is cooled, then transferred to a 250 c.c. flask, the flask rinsed 3 times with water and the contents then neutralized with 5%  $\text{HNO}_3$  until neutral to phenolphthalein indicator. A pinch of  $\text{NaHCO}_3$  is then added and the liquid made up to a volume of 250 c.c. An adequate portion, about 50 c.c., is then taken and titrated with  $\text{N}/10 \text{ AgNO}_3$ , using  $\text{K}_2\text{CrO}_4$  as indicator. A blank is made, using 50 c.c. of alcoholic  $\text{KOH}$  neutralizing with 5%  $\text{HNO}_3$  and titrating with  $\text{AgNO}_3$ .

The number of c.c.  $\text{AgNO}_3$  minus the c.c.  $\text{AgNO}_3$  for the blank is equivalent to the amount required for the chlorides from the saponification of the  $\text{CHCl}_3$



**Spirit of Chloroform.**—Mixtures of alcohol and chloroform have long been used in medicine. The preparation in the U.S.P. consists of 60 c.c. of chloroform and 940 c.c. of alcohol; that in the British, of 50 c.c. of chloroform and sufficient alcohol of 90% strength to make 1000 c.c. Under the highly erroneous term "chloric ether" mixtures of these two ingredients in uncertain proportions have had considerable vogue in Great Britain. The term chloric ether can only be properly applied to ethyl chlorate.

The proportion of chloroform present in spirit of chloroform, "chloric ether," and similar preparations may be ascertained with accuracy by introducing into a narrow graduated tube 20 c.c. of the sample and 30 c.c. of dilute sulphuric acid (1 to 6) coloured with a little fuchsin. A cork is then inserted and the contents of the tube thoroughly shaken. When the chloroform has separated, the tube is tapped to cause floating globules to sink, and about 10 c.c. of petroleum spirit are cautiously poured on the surface of the acid. The cork is reinserted and the volume of petroleum spirit employed is carefully noted, when the contents of the tube are well mixed. After separation, the volume of petroleum spirit is again observed, when its increase will be due to the dissolved chloroform. Better results are obtainable in this way than without petroleum spirit,

but great care is necessary to avoid error from expansion or contraction through alteration of temperature. Hence, before observing the volume of petroleum spirit originally used, and again before the final reading, the tube should be immersed in a cylinder of cold water for a short time. The process gives inaccurate results when the proportion of chloroform exceeds about 30%. In such cases the method given on p. 345 should be employed.

**Methylene Dichloride.**—This substance is a powerful anæsthetic. Being more expensive than chloroform, the latter liquid is sometimes substituted and sold for the former, which it closely resembles in odour. The two substances may be distinguished by their sp. gr. and b. p. The methylene dichloride burns with a smoky flame and dissolves iodine with brown colour, whilst chloroform unmixed with alcohol burns with great difficulty, giving a green-edged flame, and dissolves iodine forming a reddish-violet liquid.

A mixture of alcohol and chloroform has been substituted for methylene dichloride. On shaking the sample with water, the alcohol would be dissolved, and the chloroform would then be recognisable by its sp. gr.

**Carbon Tetrachloride.**—This substance, originally obtained by the action of chlorine upon methane, is now usually made on the large scale by the action of chlorine upon carbon disulphide. It is a heavy, colourless, moderately volatile liquid, with an odour recalling that of chloroform. It has been suggested for use as a general anæsthetic but is not well suited for that purpose. Its important use is as a solvent and for filling fire-extinguishers. B. p.  $78.1^{\circ}$ ; sp. gr.  $1.63$ . It freezes at  $-24.7^{\circ}$ . Its applicability as a solvent, especially as a substitute for the light petroleum products and coal-tar hydrocarbons, is due mainly to its non-inflammability. The general conditions of such employment have been investigated by Baskerville and Riederer (*Jour. Ind. Eng. Chem.*, 1912, 4, 645). Its high sp. gr. is one objection, as in most industrial operations of extraction the bulk of the solvent is important. The solvent powers of carbon tetrachloride are high and of wide range. Mixtures of it with considerable proportions of alcohol give a still wider range of dissolving power.

In contact with water hydrochloric acid is formed. This is a serious happening if carbon tetrachloride is to come in contact with metals. One of us (S. S. S.) has known steel drums, in an extraction apparatus, inadvisedly used for large scale

work, and even copper equipment to be seriously attacked by hydrochloric from the interaction of water on carbon tetrachloride. Carbona has been sometimes used to clean white cotton duck shoes which were damp enough to form hydrochloric acid from the tetrachloride and this acid tendered the fabric.

AMERICAN EDITORS.

Under coined trade names, especially "carbona," impure forms of carbon tetrachloride have been for some years extensively used as household substitutes for light petroleum cleaning agents. Its employment in fire extinguishers has given rise to serious dangers in consequence of its producing when in contact with air at high temperatures, several highly toxic gases, among the principal of which are free chlorine, hydrochloric acid and carbon oxychloride. The last named, commonly called "phosgene gas," is one of the most toxic gases known, a comparatively small amount rendering air irrespirable. Its use as an asphyxiating gas in warfare is well known. Deaths have occurred to men using carbon tetrachloride for extinguishing fires. The subject has been investigated by chemists of the U. S. Bureau of Mines.

Recent investigations were made by Fieldner, Katz, Kinney and Longfellow (*J. Frank. Inst.*, 1920, **190**, 543) who, in addition to studying the effects of flame and hot surfaces upon the substance, reported on the examination of a sample of the commercial article and three samples of fire-extinguisher liquid.

The sample of commercial tetrachloride contained carbon disulphide but no chloroform. One sample of fire-extinguisher liquid contained tetrachloride and chloroform, a second contained tetrachloride and a high b. p. petroleum, and a third, tetrachloride and turpentine. (It is not stated in the report whether the substance designated "turpentine" was the oleo-resin or the ordinary oil, presumably it was the latter.)

The examinations of the several samples were made by fractional distillation and noting the odours. Carbon disulphide can probably be best detected by passing the vapour of the sample through a tube containing hot copper oxide when the sulphur will be converted into sulphur oxides, a portion of which will pass on and can be collected in an alkaline solution, and another portion retained by the copper oxide. Both portions can be completely converted into sulphate by oxidation with nitric acid and the determination made with barium in the usual way.

**Bromoform.**—This substance has comparatively little importance. It is a heavy, colourless liquid, with an odour resembling that of chloroform. The sp. gr. is given as ranging from 2.829 to 2.843 at 15°. It boils at about 149° and freezes a few degrees above the m. p. of ice. It resembles chloroform in some respects, among others in giving the isocyanide reaction, but its high sp. gr. and b. p. distinguish it.

**Iodoform.**—Iodoform is produced in many reactions for detection of alcohol, acetone and other neutral alcoholic derivatives. It is a yellow solid, distinctly crystalline, the crystals showing under moderate magnification as hexagons and six-radiated forms similar to those exhibited by snow and lead iodide. It has a powerful and disagreeable odour and marked antiseptic and germicidal powers. The m. p. of iodoform is about 117°. It is but slightly soluble in water and petroleum spirit, rather more soluble in alcohol and freely soluble in ether and chloroform. It yields the isocyanide reaction. Alkali hydroxides convert it into a formate, producing at the same time a metallic iodide. The identification of iodoform is easily made by means of its odour and characteristic crystalline form.

The determination of iodoform is made by boiling the material containing it with excess of solution of sodium hydroxide in alcohol, by which the whole of the iodine present is converted into sodium iodide. The alcohol is driven off, the residue diluted with water, slightly acidified with nitric acid, filtered, and the iodine converted into silver iodide by precipitation with silver nitrate.



# SUGARS

REVISED BY LEWIS EYNON, B.Sc., F.I.C.

Under the generic name of sugars is included a large number of substances occurring naturally in the animal or vegetable kingdom or produced from the so-called *glucosides* by the action of ferments or dilute acids.

The sugars constitute a group of closely-allied compounds, in many cases distinguishable from each other only with considerable difficulty, whilst their quantitative separation is frequently impossible in the present condition of chemistry.

As a class, the sugars are crystallisable, readily soluble in water, somewhat less soluble or wholly insoluble in alcohol, and insoluble in ether and other solvents immiscible with water.

A *sweet taste* is possessed by nearly all sugars to a greater or less extent. Glycerol and glycol have a sweet taste, and, like the sugars, are polyatomic alcohols.

In many cases the sugars exert a powerful rotatory action on a ray of polarised light, the direction and extent of the rotation being peculiar to each sugar. Hence the *optical activity* is a valuable means of estimating and differentiating sugars.

**Constitution and Classification of Sugars.**<sup>1</sup>—The sugars proper are aldehydes or ketones of hexatomic alcohols and may be obtained from these by limited oxidation or, conversely, converted into them by reduction. They are divided into three great groups, *viz.*, (1) the mono-saccharides or glucoses; (2) the di-saccharides or saccharoses and (3) the poly-saccharides; *e. g.*, starch, cellulose, etc. They

<sup>1</sup> *Nomenclature.*—To avoid confusion in the following pages, the term sucrose has been used as the more scientific term for both cane and beet sugar, whilst the term cane sugar describes the commercial article irrespective of origin. The word glucose is restricted to the commercial product from starch, and dextrose is used for the pure sugar  $C_6H_{12}O_6$ , laevulose for the corresponding ketose derived from sucrose. The di-saccharide, milk sugar is also spoken of as lactose. Maltose is applied to the di-saccharide derived from starch. The abbreviation A. O. A. C. refers to "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists" revised to November 1, 1919, and published by the Association of Official Agricultural Chemists, in the United States.

mostly contain 6, or a multiple of 6, atoms of carbon, and hydrogen and oxygen in the proportion of 2:1.

**The Mono-saccharides.**—To this group belong the naturally occurring sugars containing 5 and 6 carbon atoms, known as pentoses and hexoses, and also the closely related synthetical sugars with 3, 4, 7, 8, and 9 carbon atoms. They are characterised by the following general properties:

1. They are easily oxidised and reduce Fehling's solution.
2. They form with phenylhydrazine and acetic acid sparingly soluble crystalline osazones.
3. Those hexoses which occur naturally undergo alcoholic fermentation with yeast.
4. They form additive compounds with hydrogen cyanide.

**The Di-saccharides.**—This group consists of sugars of the formula  $C_{12}H_{22}O_{11}$ ; *e. g.*, sucrose, lactose, maltose, melibiose and others formed by the union of two mono-saccharide residues through an oxygen atom. It may also be extended to include sugars, such as raffinose, formed by the union of three or more mono-saccharide residues. The general properties of the members of this group are:

1. They are converted on hydrolysis by mineral acids or by specific enzymes into mono-saccharides.
2. They are not directly fermentable unless first hydrolysed.

**The Poly-saccharides.**—This group includes substances of high molecular weight, of the general formula  $nC_6H_{10}O_5$ , such as cellulose, starch, glycogen and dextrin. They are amorphous substances and yield simpler saccharides or ultimately mono-saccharides on hydrolysis.

The tables on pp. 357 show the origin and leading characteristics of the more important mono- and di-saccharides.

**Isolation of Sugars.**—The quantitative analysis of complex artificial or natural carbohydrate mixtures is one of the most difficult problems in organic analytical chemistry. Indirect methods have almost invariably to be employed and errors in the estimation are apt to become additive.

The general methods by which sugars are isolated in the proximate analysis of animal and vegetable substances depend much on the nature of the associated substances. Principles of separation commonly utilised are: the removal of protein substances by heat or precipitation; the precipitation of dextrin and other gummy matters

MONO-SACCHARIDES

Name	Origin and mode of formation	Specific rotation	Other characters
<i>Aldohexoses</i> , $C_6H_{12}O_6$ . d-Dextrose.	Honey, sweet fruits. action of acids on starch, cellulose, etc.	By +52 7°	Very soluble, slightly sweet, reducing power marked, easily fermentable. Shows mutarotation. Forms sorbitol on reduction, gluconic acid on oxidation with bromine. Turns brown with alkalis. Forms a soluble phenylhydrazine and an insoluble osazone. Forms alkyl glucosides hydrolysed by enzymes.
d-Mannose	Ivory nuts.	+14°	Similar to dextrose. Forms an insoluble phenylhydrazine and the same osazone as dextrose. Forms alkyl glucosides not affected by enzymes.
d-Galactose.	Action of acids on milk-sugar, gums.	+81°	Less soluble. Fermented with difficulty and not by all yeasts. Shows mutarotation. Yields dulcitol on reduction and mucic acid on oxidation with nitric acid.
<i>Kelohexoses</i> , $C_6H_{12}O_6$ . d-Lævulose.	Honey, fruits, inulin.	-92°	More soluble than dextrose. Crystallises with difficulty. Easily fermented. Properties similar to dextrose. Forms a methyl phenyl-osazone. Shows mutarotation.
d-Sorbose.	Ripe mountain ash berries.	-42 9°	Not fermentable by yeast. Yields sorbitol on reduction.
<i>Aldopentoses</i> , $C_5H_{10}O_5$ . l-Arabinose	Action of acids on gums.	+105°	Not fermented. Shows mutarotation. Forms arabinic acid on oxidation, arabitol on reduction. Yields furfuraldehyde on heating with hydrochloric acid.
l-Xylose.	Action of acids on straw.	+19°	Similar to arabinose. Forms xylonic acid on oxidation and xylitol on reduction. Shows mutarotation.

## DI-SACCHARIDES

Name	Origin and mode of formation	Specific rotation	Other characters
<i>Non-reducing</i> , $C_{12}H_{22}O_{11}$ . Sucrose.	Sugar cane, beet, maple, etc.	+66.5°	Very soluble in water. Forms oxalic and saccharic acids on oxidation. Chars with conc. $H_2SO_4$ . Very easily hydrolysed by dilute acids and by invertase to a mixture of dextrose and laevulose. Fermented by yeast only after inversion. Does not show mutarotation.
Trehalose.	Trehala manna, Fungi; <i>e. g.</i> , <i>Aspergillus niger</i> .	+197°	Hydrolysed by trehalase to two molecules of dextrose. It does not exhibit mutarotation or form an osazone.
<i>Reducing</i> , $C_{12}H_{22}O_{11}$ . Maltose.	Starch, by the action of diastase or dilute acids.	+138.3°	Less soluble than dextrose. Exhibits mutarotation. Forms an osazone soluble in hot water. Hydrolysed by acids and more readily by maltase to two molecules of dextrose. Fermented by yeast enzymes after inversion.
Lactose.	Milk of mammals.	+55.3°	Less soluble than maltose and forms mucic acid on oxidation, otherwise very similar. Hydrolysed by lactase to dextrose and galactose. Not fermented by ordinary yeast. Shows mutarotation.
Melibiose.	Raffinose, by the action of acids, or top yeast.	+143°	Mutarotates. Hydrolysed by melibiase to dextrose and galactose. Fermented by bottom but not by top yeasts.
Turanose.	Melecitose, by the action of acids.	+71.8°	Hydrolysed by acids to dextrose and laevulose. Forms a phenyl-osazone. Not hydrolysed by invertase.
TRI-SACCHARIDE. <i>Non-reducing</i> , $C_{18}H_{32}O_{16}$ . Raffinose.	Sugar beet.	+104°	Does not form a hydrazone or osazone nor mutarotate. Fermented after hydrolysis. Converted into sucrose and galactose by emulsin, and into laevulose and melibiose by invertase.

by alcohol; the removal of organic acids and various other matters by basic lead acetate; concentration of the solution with a view to promoting crystallisation; and the detection and estimation of the sugars present by their reactions as reducing agents, and their relations to polarised light. A third mode of determination is based on the sp. gr. of the saccharine solution. Other useful processes for estimation or differentiation are based on the behaviour of the sugars with yeast, and on treatment with concentrated and dilute acids.

Phenylhydrazine is of great value as a qualitative reagent and the asymmetrically di-substituted hydrazines may in some cases be used with advantage. When dealing with sugar solutions it is important to avoid carefully the presence of alkali.

**Action of Concentrated Acids on Sugars.**—Organic acids act on sugars to form oxygen esters. In presence of suitable dehydrating agents acetic acid or acetic anhydride gives rise to fully acetylated compounds, *viz.*, the pentacetate in the case of the hexoses or the octacetate of sucrose, maltose, or lactose. Dextrose pentabenzoate may be used for the detection and isolation of dextrose, particularly in physiological fluids. The solution is shaken for an hour with 6 parts of benzoyl chloride and 48 parts of 18 to 20% sodium hydroxide for every part of dextrose, and cooled with ice. After 24 hours the pentabenzoate may be recrystallised from alcohol. It forms colourless needles; m. p., 179°.

**Nitric acid** when used in cold concentrated solution gives rise to nitric esters. When heated with dilute or moderately concentrated acid the sugars yield oxidation products, of which mucic, saccharic, tartaric and racemic acids are the most constant and characteristic. The formation of mucic acid is characteristic of galactose and also of di- or polysaccharides which contain galactose, *e. g.*, milk sugar or gums. For the estimation of galactose as mucic acid, see under Galactose, p. 471.

**Sulphuric Acid.**—Dextrose dissolves in cold concentrated sulphuric acid without any colouration, forming dextrose-sulphonic acid. This behaviour distinguishes dextrose from sucrose, which is carbonised by concentrated sulphuric acid with great facility. A strong syrup of sucrose mixed with concentrated sulphuric acid is immediately decomposed with evolution of sulphur dioxide and other volatile products, and formation of a bulky, black, carbonaceous mass.

### Action of Dilute Acids on Sugars. Hydrolysis or Inversion.—

When an aqueous solution of sucrose is heated with dilute sulphuric or hydrochloric acid, the solution increases in sp. gr., and the sugar loses its power of crystallising readily. This change in properties is attended by the assimilation of the elements of water, with formation of the mixture of dextrose and lævulose known as inverted or invert sugar:  $C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6$ . The rate of hydrolysis depends mainly on the proportion of acid used, its chemical activity, and the temperature employed in the operation. When sucrose is hydrolysed the optical activity is changed from right- to left-handed or is "inverted." The term *inversion* is often applied generally to the process of hydrolysis of the di-saccharides whether or not the same optical change be produced.

The property of undergoing hydrolysis when heated with dilute acids is common to all the di- and poly-saccharides. The following table shows the products of hydrolysis of the principal di- and tri-saccharides:

<i>Di-saccharide.</i>		<i>Mono-saccharides.</i>
Sucrose	=	dextrose and lævulose.
Lactose	=	dextrose and galactose.
Maltose	=	dextrose.
Melibiose	=	dextrose and galactose.
<i>Tri-saccharide.</i>		
Raffinose	=	lævulose, dextrose and galactose.

**Sucrose** is most readily and certainly inverted by adding to a solution containing not more than 25 grm. of the solid per 100 c.c. one-tenth of its bulk of fuming hydrochloric acid, and then heating the liquid to 70° for 10 or 15 minutes. Some operators prefer dilute sulphuric to hydrochloric acid, and heat the liquid to boiling for 5 or 10 minutes.

**Lactose** is less readily hydrolysed than sucrose, being unaffected by boiling for 10 minutes with 2 grm. of citric acid per 100 c.c. of the solution.

The differences in the readiness with which different sugars are hydrolysed is shown in the following table<sup>1</sup> in which the rate of hydrolysis by hydrochloric acid at about 70° of a number of sub-

<sup>1</sup> E. F. Armstrong, *Proc. Roy. Soc.*, 1904, 74, 188-194.

stances under identical conditions is recorded relatively to the most stable compound which is expressed as 100.

$\alpha$ Methyl-glucoside,	100
$\beta$ Methyl-glucoside,	180
$\alpha$ Methyl-galactoside,	540
$\beta$ Methyl-galactoside,	880
Salicin,	600
Lactose,	720
Maltose,	740
Sucrose,	about 900,000

To insure complete hydrolysis of carbohydrates other than sucrose, dilute solutions—preferably not above 5%—should be employed and the heating prolonged. Meissel (*Zeitsch. anal. Chem.*, 1883, 22, 114) uses 3% sulphuric acid or 5% fuming hydrochloric acid, obtaining in the former case a conversion of 98.5%. The liquid is heated in a water-bath for 3 or 4 hours. When the inverted solution of a sugar is to be decolourised by basic lead acetate or treated by Fehling's solution, the free acid contained in it should first be nearly neutralised by the addition of sodium carbonate.

**Action of Alkalis on Sugars.**—Sucrose is not attacked by dilute caustic alkalis or alkaline carbonates in the cold, and only very slowly, if at all, on heating. It is decomposed by boiling with concentrated alkaline solutions, and when fused with potassium hydroxide yields potassium oxalate and acetate and other products. Sucrose forms a few well-established compounds with bases and many with salts.

Dextrose and other mono-saccharides are readily decomposed by alkalis. When heated with sodium or potassium hydroxide, dextrose becomes brown at 60–70°, and decomposes entirely on prolonged boiling.

**Recognition of the Principal Kinds of Sugar.**—When a sugar has been isolated in a condition of tolerable purity, it may be recognised by the special characters described in the tables of properties on p. 364. The detection or identification of a sugar by its reactions is greatly simplified by applying the tests in a systematic manner.

All the substances referred to in the table are optically active. Hence it is not possible to have an inactive solution containing a notable quantity of one of the above sugars. If lævulose is present,

together with a certain proportion of one of the other sugars, the solution may exhibit no rotation at a certain temperature, but would do so on heating or cooling, owing to the marked influence of temperature on the optical activity of lævulose.

**Lævulose** always occurs in practice in the presence of more or less dextrose, and in such cases is most easily detected by the change in the optical activity of the solution on heating. Other distinctions between lævulose and dextrose will be found under "Lævulose."

**Lactose** is only met with in products derived from milk. It is peculiar in having its optical activity and cupric-reducing power increased by treatment with dilute acid, and in yielding mucic acid on oxidation with nitric acid. Physically it is distinguished from other sugars by its crystalline form and sparing solubility in cold water.

**Sucrose** is well characterised by its behaviour towards invertase in addition to tests 1, 3, 4, 5, and 6.

**Maltose** when unmixed with dextrose is distinguished from the latter by reactions 2 and 5, but if dextrose be also present only a quantitative application of tests 3, 4, 5, and 6 will suffice for the detection of maltose.

**Dextrin**, which often occurs together with maltose, may be detected in mixtures of the two by gradually adding a large excess of strong alcohol, when it is precipitated in flocks which often adhere to the sides of the beaker as a gummy mass. Dextrin is said to be unaffected in its optical activity by boiling with a concentrated alkaline solution of mercuric cyanide, by which treatment maltose and dextrose are oxidised and destroyed.

Numerous colour reactions for the identification of the more important sugars in carbohydrate mixtures have been described, most of which have the disadvantage that they are not very characteristic. A discussion of these hardly enters into the subject of commercial analysis. Fenton's test for carbohydrates<sup>1</sup> which involves the formation of  $\omega$ -bromomethylfurfuraldehyde, the merest trace of which may be detected, is of extreme delicacy. A small quantity of the sample is moistened with water, mixed with a few drops of phosphorus tribromide dissolved in toluene, and heated on a water-bath at 90–100° till it has become dark-coloured. It is then cooled, stirred with a few drops of ethyl malonate in a

<sup>1</sup> *Proc. Camb. Phil. Soc.*, 1906, 14, 24.

little alcohol and made alkaline by alcoholic potassium hydroxide. A characteristic blue fluorescence is obtained on dilution with much water.

*m*-Dinitrobenzene gives a violet colouration with aldoses and ketoses in moderately alkaline solution. This appears usually after 15 minutes in a 1% sugar solution.

In what follows, methods of analysis applicable to sugars in general are first described under their respective headings, and the application of these and other methods to the examination of particular sugars or sugar products is dealt with subsequently.

### METHODS OF ANALYSIS DEPENDING ON SPECIFIC GRAVITY OR SOLUTION DENSITY

**Specific Gravity of Saccharine Solutions.**—Solutions of equal concentrations containing different carbohydrates have approximately the same, though not strictly identical, sp. gr., and since the difference between the sp. gr. of the solution and that of water is nearly proportional to the concentration, the latter may be found more or less accurately by determining the former. Thus, a solution containing 10 gm. of sucrose per 100 c.c. has a sp. gr. of 1.038.6 at 15.5°/15.5° (referred to water as 1000), the increment of sp. gr. for each 1% of sucrose up to about 12% being 3.86, and the weight of sucrose, *C*, per 100 c.c. of a solution may be found from the formula:  $C = \frac{D - 1000}{3.86}$ , where *D* is the sp. gr. The divisor, 3.86, is accurate for sucrose solutions not exceeding 12% and may be used for carbohydrate solutions in general where high accuracy is not required, but for more exact work the appropriate divisor for the carbohydrate concerned must be employed.

Probably the most accurate values of the divisors at different concentrations for sucrose, dextrose, levulose, invert sugar, maltose, and "low," "medium" and "high" starch conversion products are those of Brown, Morris and Millar (*Trans. Chem. Soc.*, 1897, 71, 72, 275). These authors give the sp. gr. taken at 15.5° and referred to water at the same temperature. The following equations summarise the results; in the original paper the results are also given in the form of tables and curves from which the divisors corresponding with different concentrations can be read directly.

	Dextrose $C_6H_{12}O_6$	Laevulose $C_6H_{12}O_6$	Lactose $C_{12}H_{22}O_{11} + H_2O$	Maltose $C_{12}H_{22}O_{11} + H_2O$	Sucrose $C_{12}H_{22}O_{11}$	Dextrin $C_6H_{10}O_5$
1. Moisten the solid sugar with water, and stir in the cold with concentrated sulphuric acid (1.845 sp. gr.).	Not affected when pure.	Not affected when pure.	Not affected.	Slightly reddish or brownish, gradually turning darker. Slightly discoloured.	Charred.	Not affected.
2. Triturate the solid sugar with sodium hydroxide, or boil it with a 3 % solution for 1 minute.	Deep brown colouration.	Deep brown colouration.	Not affected.		Not affected.	Not affected.
3. To the neutral aqueous solution add a few drops of Fehling's solution and heat to boiling for a few minutes.	Red precipitate of $Cu_2O$ .	Red precipitate of $Cu_2O$ .	Red precipitate of $Cu_2O$ .	Red precipitate of $Cu_2O$ .	No change.	No change.
4. Hydrolyse by boiling with $\frac{1}{2}O$ of its bulk of strong sulphuric acid, neutralise with sodium hydroxide, and heat to boiling with Fehling's solution.	Red precipitate of $Cu_2O$ .	Red precipitate of $Cu_2O$ .	Red precipitate of $Cu_2O$ .	Red precipitate of $Cu_2O$ .	Red precipitate of $Cu_2O$ .	Red precipitate of $Cu_2O$ .
5. Heat the solution in boiling water for 3 minutes with 3 c.c. of a liquid containing 4 % of cupric acetate and 1 % of acetic acid ( $C_2H_4O_2$ ).	Red precipitate of $Cu_2O$ .	Red precipitate of $Cu_2O$ .	No change.	No change.	No change.	No change.
6. Observe the solution in the polarimeter.	Dextro-rotatory.	Laevo-rotatory.	Dextro-rotatory.	Dextro-rotatory.	Dextro-rotatory.	Dextro-rotatory.
7. Heat solution with dilute acid as in test 4, and observe again in polarimeter.	Dextro-rotatory power unchanged.	Laevo-rotatory power unchanged.	Dextro-rotatory power increased.	Dextro-rotatory power diminished.	Dextro-rotatory power changed to laevo-rotatory.	Dextro-rotatory power diminished.
8. Heat the solution in boiling water for 1 hour with 1 c.c. of phenylhydrazine and 1 c.c. of 50 % acetic acid and a little salt. Allow to cool (see page 359).	Yellow crystalline osazone insoluble in boiling water.	Same as dextrose.	Yellow crystalline osazone soluble in hot water.	Yellow crystalline osazone soluble in hot water.	No change.	Very soluble yellow osazones.
9. Treat the sugar with moderately concentrated nitric acid, and study the products of the oxidation (see page 359).	Saccharic acid.	Saccharic acid.	Mucic, saccharic and oxalic acids.	Saccharic and oxalic acids.	Saccharic and oxalic acids.	Oxalic acid.

$D$  is the required divisor in grams of anhydrous sugar per 100 c.c. (reputed).

$G$  is the sp. gr. at  $15.5^{\circ}$  when water at the same temperature = 1000.

For *Dextrose*,  $D = 3.848 - 0.00028 (G - 1000) - 0.0000028 (G - 1000)^2$ .

*Lævulose*,  $D = 3.946 - 0.00068 (G - 1000) - 0.0000007 (G - 1000)^2$ .

*Invert Sugar*,  $D = 3.897 - 0.00025 (G - 1000) - 0.0000004 (G - 1000)^2$ .

*Maltose*,  $D = 3.9435 - 0.00044 (G - 1000) - 0.000001 (G - 1000)^2$ .

"*High Transformation*" of starch by diastase,  $[\alpha]_D 188.6^{\circ}$ ,  $R = 20.2$ .

$D = 4.032 - 0.0006 (G - 1000)$ .

"*Low Transformation*" of starch,  $[\alpha]_D 149.7^{\circ}$ .  $R = 82.8$ .

$D = 3.9742 - 0.000403 (G - 1000) - 0.0000014 (G - 1000)^2$ .

Ling, Eynon and Lane (*Seventh Int. Congr. Appl. Chem.*, 1909, I, 137), have since re-determined the solution densities of dextrose, lævulose and maltose, for concentrations ranging from  $c = 1$  to  $c = 24$ . The results practically confirm those of Brown, Morris and Millar and are of importance because special care was taken to ensure the purity of the sugars employed. W. A. Davis also has made a series of determinations with highly purified dextrose, lævulose, sucrose and maltose; for all practical purposes the values referred to above may be taken as accurate.<sup>1</sup> It must be remembered that these data apply only to solutions of carbohydrates in a pure or nearly pure state. Raw sugar products contain mineral salts the influence of which on the sp. gr. of the solution must be allowed for.

Below are given the recent data obtained by Ling, Eynon and Lane.

Column (A) gives the weight of dry substance taken.

Column (B) gives the total weight of solution.

Column (C) gives the sp. gr. of the solution at  $15.5^{\circ}$ , referred to water at the same temperature.

Column (D) gives the grm. of sugar per 100 c.c. (reputed) (fluid grm. at  $15.5^{\circ}$ ).

Column (E) gives the divisor for calculating grm. of sugar per 100 c.c. (reputed) from the sp. gr.

<sup>1</sup> Cf. Davis and Daish, *J. Agric. Sci.*, 1913, 5, 437.

## SUGARS

## DEXTROSE

A	B	C	D	E
0.9992	48.7130	1.007.97	2.0675	3.855
1.0034	48.1702	1.008.08	2.0990	3.848
1.9994	50.4500	1.015.45	4.0243	3.839
3.0003	51.4028	1.022.90	5.9705	3.836
4.0176	51.5618	1.030.72	8.0311	3.825
4.9977	51.8332	1.038.26	10.0108	3.822
5.9990	52.5148	1.045.61	11.9444	3.819
7.0012	52.6845	1.053.38	13.9984	3.813
7.9990	52.8802	1.061.13	16.0513	3.808
9.0015	53.3225	1.068.63	18.0398	3.804
9.9804	53.6794	1.076.03	20.0182	3.798
11.0002	53.8410	1.084.06	22.1483	3.795
11.9125	53.3030	1.092.42	24.4141	3.786

## LÆVULOSE

A	B	C	D	E
1.1040	51.2864	1.008.55	2.1710	3.938
2.0121	50.9296	1.015.76	4.0130	3.927
3.0125	51.3828	1.023.54	6.0008	3.928
4.0528	52.7666	1.031.01	7.9186	3.921
3.9982	51.5144	1.031.36	8.0047	3.918
5.0020	51.9507	1.039.18	10.0044	3.916
5.9136	52.7600	1.045.83	11.7202	3.910
6.9985	52.6476	1.054.73	14.0207	3.904
8.2876	54.7342	1.062.78	16.0922	3.901
8.9952	53.2702	1.070.43	18.0753	3.896
10.0312	54.0708	1.077.80	19.9049	3.890
11.0257	54.3534	1.085.57	22.0211	3.886
11.6614	52.9094	1.093.55	24.1021	3.881

## MALTOSE

A	B	C	D	E
0.9422	50.1806	1.007.46	1.8916	3.944
1.9977	50.9160	1.015.66	3.9850	3.930
3.1074	51.3990	1.024.31	6.1926	3.926
3.8939	51.4710	1.030.54	7.7963	3.917
5.0569	52.2945	1.039.34	10.0504	3.914
5.9907	53.6860	1.045.63	11.6680	3.911
7.0032	52.8330	1.054.63	13.9795	3.908
7.9710	53.4580	1.061.78	15.8319	3.902
8.0323	52.9098	1.062.87	16.1081	3.903
8.9098	53.2972	1.069.84	17.8847	3.905
8.9132	53.2600	1.069.91	17.9052	3.904
10.0734	53.7766	1.078.76	20.2073	3.898
10.9333	53.5393	1.086.47	22.1809	3.897
11.7828	54.2558	1.092.35	23.7227	3.893

For tables showing the sp. gr. of sucrose solutions at 20° compared with water at 4° as determined by the *Kaiserliche Normal Eichungskommission*, (*Zeitsch. Ver. deut. Zuckerind.*, 1900, 50, [2] 1123). These tables are given in full in Fröhling's *Anleitung für die Zuckerindustrie*, 7th Ed., 1911, pages 87-91.

**Saccharometers.**—Various modifications of the hydrometer have been devised and used for ascertaining the sp. gr. of saccharine solutions.

Bates' brewers' saccharometer is much used for testing the strength of beer-worts, and hence it is described under "Malt."

On the Continent, Balling's saccharometer is much used. If  $B$  = degrees of Balling and  $b$  those of Bates, the indications of one instrument may be calculated to those of the other by the following formulæ:

$$B = \frac{260b}{360 + b}; \text{ and } b = \frac{360B}{260 - B}$$

The saccharometer of Brix is practically the same as that of Balling. In each, the number of degrees is identical with the percentage by weight of cane sugar in the solution.

The Brix spindle should be graduated to tenths. It is therefore desirable, for accuracy, that the range of degrees recorded by each individual spindle be as limited as possible, this end being best secured by the employment of sets consisting of not less than three spindles. The solutions should be as nearly as possible of the same temperature as the air at the time of reading, and if the variation from the temperatures of the graduation of the spindle amount to more than  $1^{\circ}$ , compensation must be made by reference to the table of corrections for temperature, page 368. This temperature should be  $17.5^{\circ}$ . Before taking the sp. gr. of a juice, it should be allowed to stand in the cylinder until all air bubbles have escaped.

*Example.*—A sugar solution shows a reading of  $30.2^{\circ}$  Brix at  $30^{\circ}$ . To find the necessary correction for the conversion of this reading to the reading which would have been obtained if the observation had been made at  $17.5^{\circ}$ , find the vertical column in the table headed  $30^{\circ}$  Brix, which is the nearest to the observed reading. Follow down this column until the number is reached which is opposite to the temperature of observation—in this case  $30^{\circ}$ . The number found, 0.92, is to be added to the observed reading.

Mohr (*Zeitsch. Spiritusind.*, 1906, 29, 25) has recalculated and coordinated the existing data relating to the sp. gr. of solutions of the different sugars. He shows the percentage by weight and the concentration in grm. per 100 c.c. for each of the sugars with the corresponding sp. gr. of sucrose solutions of the same concentration at the same temperature and the percentage by weight of sucrose in solutions of the same sp. gr.

TABLE FOR CORRECTION OF THE READINGS OF THE BRIX SPINDLE WHEN THE READING IS MADE AT OTHER THAN THE STANDARD TEMPERATURE, 17.5°  
(For temperatures below 17.5° the correction is to be subtracted)

Temperature, degrees Centigrade	Degree Brix of the solution													
	0	5	10	15	20	25	30	35	40	50	60	70	75	
0	0.17	0.30	0.41	0.52	0.62	0.72	0.82	0.92	0.98	1.11	1.22	1.25	1.29	
5	0.23	0.30	0.37	0.44	0.52	0.59	0.65	0.72	0.75	0.80	0.88	0.91	0.94	
10	0.20	0.26	0.29	0.33	0.36	0.39	0.42	0.45	0.48	0.50	0.54	0.58	0.61	
11	0.18	0.23	0.26	0.28	0.31	0.34	0.36	0.39	0.41	0.43	0.47	0.50	0.53	
12	0.16	0.20	0.22	0.24	0.26	0.29	0.31	0.33	0.34	0.36	0.40	0.42	0.46	
13	0.14	0.18	0.19	0.21	0.22	0.24	0.26	0.27	0.28	0.29	0.33	0.35	0.39	
14	0.12	0.15	0.16	0.17	0.18	0.19	0.21	0.22	0.22	0.23	0.26	0.28	0.32	
15	0.09	0.11	0.12	0.14	0.14	0.15	0.16	0.17	0.16	0.17	0.19	0.21	0.25	
16	0.06	0.07	0.08	0.09	0.10	0.10	0.11	0.12	0.12	0.12	0.14	0.16	0.18	
17	0.02	0.02	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.06	
18	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	
19	0.06	0.08	0.08	0.09	0.09	0.10	0.10	0.10	0.10	0.10	0.10	0.08	0.06	
20	0.11	0.14	0.15	0.17	0.17	0.18	0.18	0.18	0.19	0.19	0.18	0.15	0.11	
21	0.16	0.20	0.22	0.24	0.24	0.25	0.25	0.25	0.26	0.26	0.25	0.22	0.18	
22	0.21	0.26	0.29	0.31	0.31	0.32	0.32	0.32	0.33	0.34	0.32	0.29	0.25	
23	0.27	0.32	0.35	0.37	0.38	0.39	0.39	0.39	0.40	0.42	0.39	0.36	0.32	
24	0.32	0.38	0.41	0.43	0.44	0.46	0.46	0.47	0.47	0.50	0.46	0.43	0.40	
25	0.37	0.44	0.47	0.49	0.51	0.53	0.54	0.55	0.55	0.58	0.54	0.51	0.48	
26	0.43	0.50	0.54	0.56	0.58	0.60	0.61	0.62	0.62	0.66	0.62	0.58	0.55	
27	0.49	0.57	0.61	0.63	0.65	0.68	0.68	0.69	0.70	0.74	0.70	0.65	0.62	
28	0.56	0.64	0.68	0.70	0.72	0.76	0.76	0.78	0.78	0.82	0.78	0.72	0.70	
29	0.63	0.71	0.75	0.78	0.79	0.84	0.84	0.86	0.86	0.90	0.86	0.80	0.78	
30	0.70	0.78	0.82	0.87	0.87	0.92	0.92	0.94	0.94	0.98	0.94	0.88	0.86	
35	1.10	1.17	1.22	1.24	1.30	1.32	1.33	1.35	1.36	1.39	1.34	1.27	1.25	
40	1.50	1.61	1.67	1.71	1.73	1.79	1.79	1.80	1.82	1.83	1.78	1.69	1.65	
50	....	2.65	2.71	2.74	2.78	2.80	2.80	2.80	2.82	2.82	2.70	2.50	2.51	
60	....	3.87	3.88	3.88	3.88	3.88	3.88	3.88	3.88	3.82	3.70	3.43	3.41	
70	....	5.17	5.18	5.20	5.14	5.13	5.10	5.08	5.06	4.90	4.74	4.47	4.35	
80	....	....	6.62	6.59	6.54	6.46	6.38	6.30	6.26	6.06	5.82	5.50	5.33	
90	....	....	8.26	8.16	8.06	7.97	7.83	7.71	7.58	7.30	6.96	6.58	6.37	
100	....	....	10.01	9.87	9.72	9.56	9.39	9.21	9.03	8.64	8.22	7.76	7.42	

## METHODS OF ANALYSIS DEPENDING ON OPTICAL ACTIVITY

**Specific Rotatory Power of Sugars.**—The specific rotatory power of a sugar or other carbohydrate is best determined with a polarimeter graduated in circular degrees, although the special forms of polarimeter known as saccharimeters, which are generally used in technical sugar analysis, may also be employed; the readings obtained with the latter type of instrument are convertible into circular degrees by means of the appropriate factor (see page 372). The principles involved in the construction of polarimeters are explained in the introduction to this volume.

Sodium light is generally used for measuring the specific rotation of optically active substances, and the specific rotation,  $[\alpha]_D$ , is

given by the expression,  $\frac{\alpha_b}{lc}$ , where  $\alpha$  is the observed angle of rotation caused by a column of solution,  $l$  decimetres in length, containing  $c$  grm. of dissolved substance per 100 c.c. Since the specific rotation of sugars is sensibly affected in most cases by the concentration and temperature of the solution, both factors should be stated as a general rule.

The specific rotations of the more important sugars, in aqueous solution of about 10% concentration at  $0^\circ$ , are given in the following table, those of the rarer sugars being shown in the table on pp. 357, 358.

Sugar	$[\alpha]_D$
<i>d</i> -Dextrose . . . . . $C_6H_{12}O_6$	$+52.7^\circ$
<i>d</i> -Galactose . . . . . $C_6H_{12}O_6$	$+81^\circ$
<i>d</i> -Lævulose . . . . . $C_6H_{12}O_6$	$-92^\circ$
Sucrose . . . . . $C_{12}H_{22}O_{11}$	$+66.5^\circ$
Maltose . . . . . $C_{12}H_{22}O_{11}$	$+138.3^\circ$
Lactose . . . . . $C_{12}H_{22}O_{11}$	$+55.3^\circ$
Raffinose . . . . . $C_{18}H_{32}O_{16}$	$+104.5^\circ$

Tollens gives the following formulæ for calculating the specific rotation of solutions of sucrose containing from 18 to 69% of the sugar:

$$[\alpha]_D^{20^\circ} = 66.386 + 0.015035p - 0.0003986p^2$$

and of solutions containing from 4 to 18% of the sugar:

$$[\alpha]_D^{20^\circ} = 66.810 - 0.015553p - 0.000052462p^2$$

where  $p$  is the weight of sugar per 100 grms. of solution.

The same authority gives the following formula for calculating the specific rotation of solutions of dextrose:

$$[\alpha]_D = 52.5 + 0.018796p + 0.00051683p^2$$

In the earlier literature, specific rotation is frequently expressed in terms of the "mean yellow" ray,  $[\alpha]_y$ , as measured with the transition tint polarimeter. The transition tint, however, is not a ray of definite refrangibility, and transition tint instruments are now seldom or ever used since it is difficult to obtain concordant results.

To convert values for  $[\alpha]_j$  to those for  $[\alpha]_D$  the former must be multiplied by 0.9.

In the case of saccharimeters, commonly used in sugar analysis, the rotation of the plane of polarisation is compensated by an adjustable quartz wedge or system of wedges, and these instruments, owing to the fact that the rotation dispersions of quartz and carbohydrate solutions are practically the same, can be used with ordinary white light, whereas polarimeters graduated in circular degrees can only be used with monochromatic light, *e. g.*, that given by the sodium flame.

Saccharimeters, or quartz compensation instruments, are provided with a scale and vernier for reading percentages of sucrose directly when a solution of certain concentration is examined in a 200 mm. tube. Two such sugar scales are in common use, *viz.*, the Ventzke or German scale which is employed, for instance, in saccharimeters made by Schmidt & Haensch, and the French scale, used in saccharimeters of French manufacture. That quantity of sucrose which when dissolved in water, made up to 100 c.c., and read in a 200 mm. tube, indicates 100° of the scale is known as the "normal" weight for the instrument in question. The Ventzke normal weight originally employed was 26.048 grm. dissolved in 100 Mohr's c.c. (giving a solution of sp. gr. 1.1 at 17.5°/17.5°), whilst that employed with French instruments was 16.35 grm. (Soleil-Duboscq), or 16.27 grm (Laurent). Owing to the want of uniformity due to the use sometimes of Mohr's c.c. and sometimes of metric c.c., the International Commission for Uniform Methods of Sugar Analysis decided, at its meeting in Paris in 1900, that (for the Ventzke scale) 26.00 grm. should in future be adopted as the normal weight for 100 metric c.c. at 20°, that all measuring vessels should be graduated in true metric c.c. at this temperature, and that the rotation of chemically pure sucrose should be taken as the basis for saccharimetry. The Commission recommended the following method for the preparation of chemically pure sucrose:

Prepare a hot, saturated, aqueous solution of the purest commercial sugar, precipitate the sugar with absolute ethyl alcohol, spin the sugar carefully in a small centrifugal machine, and wash, in the latter, with absolute alcohol. Re-dissolve the sugar in water, again precipitate the sugar from saturated solution with alcohol and wash as before. Dry the second crop of crystals between blotting paper and preserve in glass vessels for use. Determine the moisture still con-

tained in the sugar and take this into account when weighing the sugar which is to be used.

The preparation of the normal sugar solution for the Ventzke or German scale is as follows:

26 grm. of chemically pure sugar, prepared as above described, are weighed in air with brass weights, dissolved in water at  $20^{\circ}$ , in a flask graduated to contain 100 metric c.c.; the solution is made up to the mark, well mixed, filtered if necessary, and polarised in a 200 mm. tube at  $20^{\circ}$ . The saccharimeter must, under these conditions, indicate 100 units on the scale, and each scale division correspond to 0.2600 grm. of sucrose. The scale division readings are frequently referred to as "degrees Ventzke" or " $^{\circ}$  V."

In tropical countries, where the laboratory temperature is commonly higher than  $20^{\circ}$ , saccharimeters may be graduated at any suitable temperature under the conditions specified above, provided that the analysis of the sugar be made at the same temperature, *i. e.*, that the volume be completed and the polarisations made at the temperature of graduation.

The saccharimeter scale can be checked at various points by means of standardised quartz plates which are supplied by the makers of the instruments. An alternative method of control consists in weighing various quantities of pure sucrose, preparing solutions thereof and polarising under the conditions described above. In checking and using the saccharimeter Schönrock (*Zeitsch. Ver. deut. Zuckerind.*, 1904, **54**, 521) proposes that a solution (6%) of potassium dichromate. 1.5 cm. in thickness, should be interposed between the source of light and the saccharimeter; this has the effect of cutting off the light of shorter wave-length, and thus rendering the rotatory dispersion of the filtered light more nearly equal for quartz and sugar solutions.

With regard to the French scale it was decided by the International Congress of Applied Chemistry in 1896 that the normal weight of sucrose for saccharimeters of this type should be 16.29 grm. and that the solution should be made up to 100 metric c.c. at  $20^{\circ}$ .

According to C. A. Browne (*Handbook of Sugar Analysis*, 1912 Ed., p. 145), the relations between angular degrees (sodium light or *D* line), the Ventzke sugar scale for the normal weight of 26 grm. and the French sugar scale for the normal weight of 16.29 grm. are as follows:

<i>Scale</i>	<i>Equivalent</i>
1° Ventzke sugar scale	= 0.34657° angular rotation <i>D</i> .
1° Angular rotation <i>D</i>	= 2.88542° Ventzke sugar scale.
1° French sugar scale	= 0.21666 angular rotation <i>D</i> .
1° Angular rotation <i>D</i>	= 4.61553° French sugar scale.
1° French sugar scale	= 0.62516° Ventzke sugar scale.
1° Ventzke sugar scale	= 1.59960° French sugar scale.

The relation between degrees Ventzke and angular rotation *D* is not quite the same for the different sugars; the following factors are given by Brown, Morris and Millar (*Trans. Chem. Soc.*, 1897, **71**, 92) for converting Ventzke to angular degrees *D*:

Sucrose,	10% solution.....	0.3469
Maltose,	10% solution.....	0.3449
Maltose,	5% solution.....	0.3457
Dextrose,	10% solution.....	0.3442
Dextrose,	5% solution.....	0.3454
Starch products,	10% solution.....	0.3458
Starch products,	5% solution.....	0.3454

In the analysis of mixtures of sugars or of starch conversion products, the polarimetric or saccharimeter readings must of course be taken in conjunction with data obtained by other methods such as copper reduction and fermentation. The following table compiled by Morris (*J. Inst. Brewing*, 1898, **4**, 174) will be found of great use in the analysis of such mixtures; the figures apply to 10% solutions at 20°.

	$[\alpha]_D$	Reading in a 200 mm. tube in the half shadow polarimeter employing white light. Ventzke divisions for 1 gm. of sugar in 100 c.c.
Dextrin.....	202.0°	11.66
Maltose (anhydrous)...	138.0°	7.97
Sucrose.....	66.5°	3.84
Dextrose.....	52.8°	3.05
Lævulose.....	-92.0°	-5.31
Invert sugar.....	-19.6°	-1.13

It is now recognised that a rise of temperature occasions a lowering of the rotation of sucrose. To correct for this Watts and Tempany give the formula: Polarisation  $-0.00031 tN$  where  $N$  is the Ventzke scale reading and  $t$  the difference between the temperature of observation and that at which the instrument was standardised.

The following precise instructions regarding the care of the instruments used are given by the A. O. A. C.:

In effecting the polarisation of substances containing sugar employ only half-shade or triple field instruments.

During the observation keep the apparatus in a fixed position and so far removed from the source of light that the polarisation nicol is not warmed. Make several readings and take the mean thereof, but no one reading may be neglected.

In making a polarisation use the whole normal weight for 100 c.c., or a multiple thereof for any corresponding volume.

As clarifying and decolorising agents use either basic lead acetate, alumina cream, or concentrated solution of alum. Bone-black and similar decolorising agents are to be excluded.

After bringing the solution exactly to the mark at the proper temperature and after wiping out the neck of the flask with filter-paper, pour all of the well-shaken clarified sugar solution on to a rapidly acting filter. Reject the first portions of the filtrate and use the rest, which must be perfectly clear, for polarisation.

**Polarimetric Estimation of Sucrose in the Absence of Other Optically Active Substances.**—It is evident, from what has been said above, that the direct polarimetric or saccharimeter reading only represents the percentage of sucrose in a substance when the latter is free from other optically active constituents. Thus, the direct saccharimeter reading indicates the sucrose-content of raw beet sugars and beet juices provided that these are practically free from other active substances such as invert sugar (as is generally the case). It does not, however, represent the true sucrose-content of raw cane sugars, or cane or beet molasses or syrups, since these materials contain a greater or less proportion of invert sugar; the difference between saccharimeter reading and sucrose-content is comparatively small in the case of high-grade raw cane sugars but is large in the case of molasses and syrups. Raw cane sugar is frequently bought and sold on the basis of "polarisation" only, however, and it must be remembered that the "polarisation" (*i. e.*

saccharimeter reading) is a conventional figure only which affords an agreed basis between the buyer and seller, and indicates approximately the value of the sugar.

Since most commercial sugar products yield solutions which are too highly coloured to be readable in the saccharimeter, it is generally necessary to decolorise the solutions with clarifying or defecating agents, of which basic lead acetate solution and alumina cream are most commonly employed.

**1. Basic Lead Acetate Solution.**—Prepare by boiling 430 grm. of neutral lead acetate, 130 grm. of litharge and 1000 c.c. of water for half an hour. Allow the mixture to cool and settle and dilute the supernatant liquid to 1.25 sp. gr. with recently boiled water. Solid basic lead acetate may be substituted for the normal salt and litharge in the preparation of the solution.

**2. Alumina Cream.**—Prepare a cold saturated solution of alum in water and divide into two unequal portions. Add a slight excess of ammonia to the larger portion and then add by degrees the remaining alum solution until a faintly acid reaction is secured. The precipitated alumina is washed with water by decantation until practically free from salts.

**Preparation of the Solution of Sugar for the Polarimeter.**—The standard quantity of the sample is weighed out, introduced into a 100 c.c. flask and dissolved in about 50 c.c. of water. If this solution be clear and colourless it is diluted to 100 c.c. and introduced into the tube of the polarimeter. If the liquid is coloured to any notable extent, as is usually the case with commercial sugars, it has first to be decolorised. This clarification may be effected by means of either of the reagents noted above. It is advisable to reject the first runnings. The following method of clarification is very efficacious even under extremely unfavorable conditions: The normal quantity of sugar is dissolved in about 50 c.c. of water in a flask holding 100 c.c. According to the quality of the sample the solution will be (1) colourless but cloudy, (2) yellow or brown, or (3) almost black. In the first case, add about 3 c.c. alumina cream and 1 drop of basic lead acetate solution. In the second case, the same volume of alumina cream may be used, but the lead solution increased to 0.5 to 5 or even 10 c.c. In the third case add about 2 c.c. of a 10% solution of sodium sulphite, and then the lead solution gradually, with constant shaking, till no further precipitate is

produced. Whichever mode of clarification is adopted, the liquid is well agitated and allowed to stand at rest for a few minutes, to insure the complete separation of any precipitate. The flask is then filled nearly to the mark with water, and the froth allowed to rise to the surface, when it is destroyed by the cautious addition of a few drops of spirit or a single drop of ether. Water is then added exactly to the mark, the contents of the flask thoroughly mixed by agitation, and the liquid filtered through a dry filter.

The validity of the simple direct polarimetric reading of a standard solution of a commercial sugar as a measure of the actual amount of sucrose contained in it is the most vexing question of industrial sugar chemistry and the attention of chemists has been largely directed towards securing uniformity in the process. It is out of the question here to do more than briefly refer to some of the difficulties, especially as much that has been written on the matter is of a controversial nature.

The amount of basic lead acetate solution required for clarifying solutions of raw sugars seldom exceeds 4 or 5 c.c. for 26 grm. of sample, and does not cause any serious error either in the direct polarisation or in the polarisation after inversion. In the case of low-grade products such as molasses, however, which contain considerable proportions of mineral salts and organic non-sugar substances, much larger quantities of the lead solution are necessary for clarification, and the saccharimeter reading is thereby liable to be vitiated by certain errors, *viz.*: (1) that due to the volume of the lead precipitate; (2) that due to the influence of basic lead acetate on the rotatory power of sucrose; (3) that due to precipitation of reducing sugars, and especially of *lævulose*, by the basic lead acetate.

With regard to the volume of the lead precipitate, it is obvious that this decreases the volume of solution and tends therefore to increase the saccharimeter reading. The reviser (*Seventh Int. Congr. Appl. Chem.*, London, 1909) has shown that even with a very low-grade sugar (polarising about  $73^\circ$ ) the error due to this cause is rather small (of the order of  $0.1^\circ$  to  $0.2^\circ$ ), but that the error is more serious in the case of molasses. The use of anhydrous basic lead acetate as proposed by Horne (*J. Amer. Chem. Soc.*, 1904, **26**, 186) eliminates this error. The dry powdered salt should contain 72.8% of lead which corresponds to a composition

of  $3\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2, 2\text{PbO}$ . The normal or half normal weight of the sugar sample is dissolved in water and made up to volume. Successive small quantities of the dry clarifying agent are added with shaking; excess is easily avoided since each particle of the powder added produces a precipitation and no more is added when the precipitation begins to be slight. In such cases the filtrate is free from lead and clarification involves no appreciable error. With molasses, etc., which yield a very abundant precipitate on clarification, it is advisable to add some dry coarse sand to facilitate precipitation.

The influence of basic lead acetate on the rotatory power of sucrose has been investigated by Bates and Blake (*J. Amer. Chem. Soc.*, 1907, **29**, 286), who have shown that on adding 0.5 to 5 c.c. of the lead reagent to a solution of the normal weight of sugar, the saccharimeter reading is slightly decreased (by about  $0.1^\circ$  V.) and that with larger quantities of the lead reagent the reading shows a progressive increase which amounts to  $0.95^\circ$  V. with 63 c.c. of the lead reagent, the influence being *nil* when about 6 c.c. of lead solution are employed. The effect of the basic lead acetate appears to be due to the formation of soluble lead sucates of rotatory powers different from that of sucrose.

The most serious error involved in the defecation of low grade products with basic lead acetate is that due to precipitation of reducing sugars and especially of l  vulose. That such precipitation does occur has been shown by various workers, e. g., Gill (*Trans. Chem. Soc.*, 1871, **24**, 91), Pellet (*Bull. Assoc. Chim. Sucr.*, 1891, **9**, 439) Edson (*Zeitsch. Ver. deut. Zuckerind.*, 1890, **40**, 1 037), Geerligs (*Seventh Int. Congr. Appl. Chem.*, London, 1909) and the reviser (*Seventh Int. Congr. Appl. Chem.*, London, 1909). The precipitation of l  vulose under these conditions has been questioned by W. A. Davis (*J. Soc. Chem. Ind.*, 1916, **35**, 201) who considers that this sugar is either destroyed by the basic lead acetate or transformed into another carbohydrate with a different specific rotatory power and a smaller reducing power. It has since been shown by Deer (*Int. Sugar J.*, 1916, **18**, 402) however, that if a solution of invert sugar containing sodium sulphate is treated with basic lead acetate a considerable proportion of the sugar is precipitated, and Pellet (*Bull. Assoc. Chim. Sucr.*, 1917, **35**, 186) has pointed out that in liquids such as molasses, containing a considerable amount

of salts, the lead precipitate always carries down some reducing sugar, although this may not happen with liquids containing organic matters but almost or quite free from salts. It should be pointed out that in those cases where the error due to precipitation of reducing sugars is most serious, *viz.*, with molasses, the direct polarisation alone does not indicate the sucrose-content since it is affected in greater or less degree by the reducing sugars and the method of double polarisation employed in such cases (see below) eliminates the error caused by precipitation of lævulose by the lead reagent.

**Polarimetric Estimation of Sucrose in Presence of Glucose, Invert Sugar or Other Reducing Sugars.**—As stated in the preceding section, the direct polarisation only indicates the true sucrose-content when the material under examination is practically free from optically active substances other than sucrose. To determine the sucrose-content of materials such as syrups and molasses or raw cane sugar which contain invert sugar, recourse must be had to the method of double polarisation. This method depends on the fact that when sucrose is heated with a dilute mineral acid or other suitable hydrolyst, it undergoes hydrolysis with the formation of equal quantities of dextrose and lævulose. Since sucrose is dextrorotatory and the mixture of dextrose and lævulose is lævorotatory, the reaction is frequently termed “inversion” and the product is termed “invert” sugar. The change in rotatory power is a measure of the amount of sucrose originally present. Hydrochloric acid is generally used for effecting hydrolysis and the process devised by Clerget and since modified by Herzfeld (*Zeitsch. Ver. deut. Zuckerind.*, 1888, **38**, 699) is as follows: Half the normal weight (13 grm.) of sucrose or of the sample is dissolved in 75 c.c. of water in a 100 c.c. flask and 5 c.c. of 38% hydrochloric acid are added. The flask, provided with a thermometer, is heated in a water-bath, with continuous shaking, the rate of heating being so regulated that the solution attains a temperature of 67 to 70° in 2½ minutes. The solution is kept at this temperature for 5 minutes, the flask is then removed from the bath and rapidly cooled; the solution is made up to volume, well mixed, filtered if necessary and polarised in a water-jacketed 200 mm. tube at 20°. Since half the normal weight of substance has been used the reading obtained must be multiplied by 2. With pure sucrose the doubled saccharimeter reading after inversion is  $-32.66^{\circ}$  V.

(i. e.,  $-16.33 \times 2$ ) at  $20^\circ$ , a change in rotation of  $\{100 - (-32.66)\} = 132.66^\circ$  V. Owing to the variation in the rotatory power of lævulose with temperature, this factor varies according to the temperature of the inverted solution and the percentage of sucrose,  $S$ , is calculated from the general formula:

$$S = \frac{100D}{142.66 - \frac{t}{2}}$$

in which  $D$  is the change of rotation and  $t$  the temperature at which the saccharimeter reading of the inverted solution is taken, the value 142.66 being the change of rotation of pure sucrose at  $0^\circ$ . It is essential that the direct and inversion polarisations should be made at the same temperature; otherwise the rotation due to the lævulose present in the original material will differ with the two readings and error will occur. It is advisable that the two readings should also be taken as near  $20^\circ$  as possible, though divergence from this temperature, within  $1$  or  $2^\circ$  does not cause appreciable error. As a general rule it is unnecessary to weigh out a separate half normal portion of the sample for the inversion; instead of this 50 c.c. of the normal sugar solution that has been prepared for direct polarisation are diluted to 75 c.c. with water and further treated as above described. In most cases the amount of lead in the filtered solution that has been used for direct polarisation is insufficient to interfere with the inversion (see, however, p. 387).

The official A. O. A. C. method is substantially the same as that above described save in the following particulars. The normal clarified solution that has been used for direct polarisation is freed from lead either (1) by adding anhydrous potassium oxalate a little at a time, avoiding excess and removing the precipitated lead by filtration, or (2) by adding anhydrous sodium carbonate under the same conditions. 50 c.c. of the lead-free filtrate (neutralised with a few drops of dilute hydrochloric acid if sodium carbonate was used for removing the lead) are diluted to 75 c.c. with water and treated with 5 c.c. of hydrochloric acid (sp. gr. 1.20) little by little, the flask being rotated during this addition. The inversion is carried out as above described excepting that when the solution has been heated to  $67$  to  $69^\circ$  (in  $2\frac{1}{2}$  to 3 minutes) it is kept at this temperature for 7 to  $7\frac{1}{2}$  minutes instead of for 5 minutes.

The A. O. A. C. also gives the following alternative method: (1) To 50 c.c. of the clarified solution, freed from lead, add 5 c.c. of hydrochloric acid (sp. gr. 1.20) and set aside for 24 hours at a temperature not below 20°, or (2) if the temperature be above 25° set aside for 10 hours. Make up to 100 c.c. at 20° and polarise.

In spite of the numerous researches which have been carried out during the past 20 years with the object of standardising the method of estimating sucrose by inversion, it is now generally recognised that the Clerget-Herzfeld process is not entirely satisfactory in many cases. This is especially true of the estimation of sucrose in the mother liquors and molasses of sugar factories. In such cases, many possibilities of error arise. These have been recently summarised by H. Pellet in a monograph *Dosage du Sucre par Inversion* originally published in *La Sucrerie Indigène*, 1913, which forms a valuable survey of the whole question. The following is a brief summary of the principal sources of error in this method.

1. It is necessary to make allowance for the change in the specific rotation of sucrose caused by dilution. In the ordinary Clerget-Herzfeld formula the constant used, *viz.*, 142.66 is correct only when the quantity of sucrose present is the half-normal weight, namely, 13 grm.<sup>1</sup> In the case of products such as crude sugars and molasses, in which the actual sugar may be only 45%, it is necessary to use the constant which applies to the particular concentration of the sucrose actually present. This may be obtained from the following table, due to Herzfeld in 1888; or it can be determined directly by making a control observation with a solution of pure sucrose of approximately the same concentration as that present in the actual analysis. The latter method is really preferable because in this way any error in the instrument or in the graduation of vessels, etc., is allowed for.

2. The principal source of error in estimating sugars in beet molasses, or in vegetable extracts in general, is due to the presence of amino acids or acid amides (such as glutamine, glutamic acid, asparagine and aspartic acids), which have a decided specific rotatory power; the error arises in the ordinary inversion process owing to the fact that such substances have a very different specific rotation in aqueous solution, in presence of basic lead acetate and in presence

<sup>1</sup> Stanek (*Zeitsch. Zuckerind. Böhm.*, 1914, 38, 289), states that the constant 142.66, for this concentration, strictly holds good only when the inverted solution is polarised within 3 to 5 minutes; if the examination is delayed, as for instance, when the solution has to be decolorised, for 15 to 30 minutes the value 142.66 is too low, and the constant 143 should then be used.

TABLE SHOWING  
ALTERATION OF HERZFELD CONSTANT WITH CONCENTRATION  
OF SUCROSE

Grams sucrose per 100 c.c.	Constant in Herzfeld formula	Grams sucrose per 100 c.c.	Constant in Herzfeld formula
1	141.85	11	142.52
2	141.91	12	142.59
3	141.98	13	142.66
4	142.05	14	142.73
5	142.12	15	142.79
6	142.18	16	142.86
7	142.25	17	142.93
8	142.32	18	143.00
9	142.39	19	143.07
10	142.46	20	143.14

of hydrochloric acid. Pellet gives data for the amides named above, which show that in some cases, for example that of glutamic acid, a solution which is strongly levorotatory in presence of a slight excess of basic lead acetate becomes strongly dextrorotatory when the solution is made acid. Consequently, the difference between the direct reading, taken in presence of basic lead acetate, and that obtained in acid solution, after inversion by the Herzfeld process, does not in such cases give a true measure of the sucrose present; the change of rotation found is less than that actually due to the sugar present, which is consequently returned at a lower figure than is actually correct. There is also an error due to the transformation of an amide such as glutamine or asparagine into an acid such as glutamic acid, by partial hydrolysis, this change being accompanied by a change of rotation. Pellet found that these errors are largely eliminated by taking the direct polarisation reading in sulphurous acid solution.

Ogilvie (*Int. Sugar J.*, 1912, 14, 624) has shown that the original Pellet method gives low results for sucrose owing to an insufficiency of sulphur dioxide being used and he has modified the method as follows:

**Ogilvie's Modification of Pellet's Method of Estimating Sucrose in Molasses.**—200 c.c. of a normal sugar weight solution of the sample, defecated with a slight excess of basic lead acetate solution, and filtered, is prepared. For the direct reading, 50 c.c. of this solution are transferred to a 100 c.c. flask, saturated with sulphur

dioxide, the flask being immersed in cold water, and made up to volume with water at standard temperature. Or, instead of saturating the liquid with sulphur dioxide, the 50 c.c. may be made up to 100 c.c. with a freshly prepared solution of sulphurous acid containing about 7.5 grms. of sulphur dioxide in 100 c.c. After mixing the liquid treated with sulphurous acid, and adding a little decolorising carbon if necessary, it is filtered and polarised at  $20^{\circ}$  in a 200 mm. water-jacketed tube. For the inversion reading 50 c.c. of the same defecated and filtered normal solution of the molasses as that used for the direct reading are transferred to a 100 c.c. flask, 25 c.c. of water and 5 c.c. of hydrochloric acid (1.19 sp. gr.) added, the liquid inverted according to the Herzfeld procedure, and read in the same 200 mm. water-jacketed tube at  $20^{\circ}$ . Finally the percentage of sucrose is calculated from the two readings in the ordinary way, applying, however, the constant from the Herzfeld table appropriate to the particular concentration used. It is advisable, in operating the inversion polarisation especially when examining very impure products, to carry out the determination in duplicate, continuing heating at  $69^{\circ}$  in the second test for 5 minutes longer than in the first. In this way the complete inversion of the sucrose in presence of an abnormally high ash and non-sugar content is insured. Since the half-normal weight is used for the direct as well as for the inversion polarisation, both readings must of course be doubled in making the calculation.

The Clerget method is applicable to the estimation of sucrose only so long as other sugars, inulins, starches and glucosides, which are also inverted by acids, are not present. In other cases invertase may be used to effect hydrolysis.

**Preparation of Invertase.**—Invertase is a soluble enzyme present in yeast and very widely distributed in plants. It has the property of rapidly and completely effecting the transformation of sucrose into invert sugar but is entirely without action on dextrose, lævulose, maltose or lactose. Indeed, the only other substances which are hydrolysed by invertase are tri-saccharides like raffinose and gentianose which contain sucrose in their molecule.

Invertase is most conveniently prepared as follows. Dry, pressed yeast is crumbled as finely as possible and spread out in a thin layer on a sheet of porous paper in a dry, airy place; in the course of a day or two it dries to a light friable powder. When perfectly dry,

it may be bottled and kept for 12 months or more without much loss of activity. 5 grm. of this are shaken for an hour with 100 c.c. of water containing 0.5 c.c. of toluene, and filtered clear. A few c.c. of this solution are added to the saccharine solution under examination, a little toluene is added and the mixture incubated in a corked vessel preferably at a raised temperature—37° to 50°—for a few hours. The determination of the optical rotatory activity and reducing power in the inverted solution is carried out in the ordinary manner, due allowance being made for the volume of enzyme solution added. This need only be 1 c.c. or less if the amount of sucrose to be inverted is small and at the higher temperature the time required for complete inversion may be less than an hour.

Kjeldahl employed a little fresh washed yeast in the presence of thymol to effect inversion, fermentation being prevented by the antiseptic.

The use of chloroform or ether as an antiseptic is in general undesirable. The former must be got rid of by heating the liquid after inversion, as it exerts a cupric reducing action. Ether, unless very pure, may adversely affect the enzyme. Invertase may also be prepared by allowing brewer's yeast to liquefy—this takes a few days at 37°. The filtered liquid has a high hydrolytic power. The enzyme may be partially purified by precipitation with alcohol and redissolution of the precipitate in a minimum of water. This extract keeps well in presence of toluene in closed vessels in the dark.

The use of invertase instead of hydrochloric acid as hydrolyst has been investigated by Ogilvie (*J. Soc. Chem. Ind.*, 1911, 30, 62; *Int. Sugar J.*, 1912, 14, 89) in connection with the estimation of sucrose in low-grade products such as molasses. Since the action of invertase is prevented by the presence of basic lead acetate, the latter must first be removed, and Ogilvie found that this is best effected by precipitating the lead as sulphite. The procedure is as follows: Four times the normal weight of the sample are transferred to a 200 c.c. flask, defecated with the minimum amount of basic lead acetate solution and a little alumina cream is added, the whole being made up to volume, shaken and filtered. 100 c.c. of the filtrate are transferred to a small beaker, sulphur dioxide passed in from a cylinder of the liquefied gas till a faint odour is perceptible (the indication that all the lead is precipitated), then the liquid transferred to a 200 c.c. flask, made up to the mark and well mixed. Sufficient cal-

cium carbonate (dried) in fine powder, to neutralise the excess of acidity, and a little recently ignited kieselguhr are added, the solution then being filtered. In this way a normal solution is obtained which is sufficiently clarified to give a distinct polarimetric reading, is free from lead and excess of acidity, and is therefore well suited for the invertase inversion. For this, 50 c.c. of the molasses solution, prepared as just described and contained in a 100 c.c. flask, are raised in a constant temperature bath to 50 to 55°, when 0.5 grm. of washed brewery yeast (top fermentation) and 2 drops of acetic acid are added, and the temperature maintained as near 55° as possible for 4½ to 5 hours. At the end of this time, the liquid is cooled, alumina cream or a little kieselguhr added to assist filtration, and made up to volume at standard temperature. The clear filtrate is then polarized in a water-jacketed tube at 20°, the temperature being determined by a thermometer reading to 0.1°. The inversion factor for the invertase inversion at 20° was determined by Ogilvie on 50 c.c. of a half-normal sugar weight solution of pure sugar, *i. e.*, 6.5 grms. of sugar dissolved to 50 c.c. and made up after inversion to 100 c.c. The value obtained was 131.6 at 20° which is somewhat lower than the factor for hydrochloric acid inversion with the same concentration of sugar.

The official A. O. A. C. methods of preparing the invertase solution and carrying out the invertase inversion are as follows:

**Invertase Solution.**<sup>1</sup>—Mix 1 kilo. of pressed bakers' or brewers' yeast with 1 litre of tap water and 50 c.c. of toluene and keep at room temperature 2 to 3 days to allow autolysis to proceed to the stage of maximum inverting activity. Then add neutral lead acetate in slight excess, filter, precipitate the lead in the filtrate with hydrogen sulphide, filter again and then dialyse the filtrate thoroughly in a collodion sac. Preserve in an ice box the dialysed solution with the addition of a little toluene to prevent the growth of micro-organisms. Note the optical activity of the invertase solution and correct the invert reading according to the amount of the solution used.

**Invertase Inversion.**—Dissolve the normal weight (26 grm.) of the substance in water, clarify, make up to volume and take the direct polarisation (*P*). If lead has been used as a clarifying agent, remove the excess of lead from the filtrate with anhydrous sodium carbonate or potassium oxalate and filter. To 50 c.c. of the filtrate in

<sup>1</sup> C. S. Hudson, *J. Amer. Chem. Soc.*, 1914, 36, 1566.

a 100 c.c. flask, add acetic acid, drop by drop, until the reaction is acid to litmus, add 10 c.c. of the invertase solution, fill the flask with water nearly to 100 c.c. and let stand in a warm place (about 40°) overnight. Cool and make up to 100 c.c. at 20°. Polarise at 20° in a 200 mm. tube. Allow the solution to remain in the tube for an hour and repeat the polarisation. If there is no change from the previous reading, the inversion is complete, whereupon the reading and temperature of the solution are carefully noted. Correct the reading for the optical activity of the invertase solution and then multiply by 2. Calculate the percentage of sucrose by the following formula:

$$S = \frac{100(P-I)}{142 - \frac{T}{2} - 0.0065 \left[ 142 - \frac{T}{2} - (P-I) \right]}, \text{ in which}$$

$S$  = percentage of sucrose

$P$  = direct reading

$I$  = invert reading

$T$  = temperature at which invert reading is made.

Ogilvie has shown that invertase gives practically the same results for molasses as Pellet's sulphurous acid method and both values are in close agreement with the values found by the Andrlik method *Zeitsch. Zuckerind.*, Böhm, 1906, 31, 417) in which hydrochloric acid is used and urea is added to stop the invertive action of the acid on the sucrose.

#### CANE MOLASSES

	Cuban molasses		Egyptian molasses	Javan molasses	American syrup
	No. 1	No. 2			
Ash (sulphated) . . . . .	6.77	7.34	10.92	10.97	6.05
Reducing sugars . . . . .	18.71	18.56	11.70	21.98	26.55
1. Alkaline (basic lead acetate) polarisation . . . . .	32.20	31.40	39.50	34.30	39.70
2. Neutral direct polarisation . . . . .	30.50	29.90	38.10	33.46	39.65
3. Acid (HCl+urea) polarisation (Andrlik) . . . . .	30.50	30.50	38.40	33.38	39.26
4. Acid (SO <sub>2</sub> ) direct polarisation (Pellet) . . . . .	30.60	30.50	38.30	33.30	39.10
5. Invertase inversion polarisation . . . . .	-16.40	-15.50	-17.90	-14.08	-11.40
6. Acid inversion (Herzfeld) polarisation . . . . .	-16.30	-15.40	-18.02	-14.06	-12.28
7. % sucrose, using invertase . . . . .	35.6	34.40	42.4	36.1	38.7
8. % sucrose, using acid as hydrolyst and the alkaline direct polarisation . . . . .	36.7	35.40	43.4	36.6	39.3
9. % sucrose, using acid as hydrolyst and the neutral direct polarisation . . . . .	35.4	34.2	42.4	35.9	39.2
10. % sucrose, using acid as hydrolyst and (HCl+urea) direct polarisation (Andrlik) . . . . .	35.4	34.7	42.6	35.9	38.9
11. Sucrose, using acid as hydrolyst and (SO <sub>2</sub> ) direct polarisation (Pellet) . . . . .	35.5	34.7	42.5	35.8	38.8

## BEET MOLASSES

(Ogilvie, *J. Soc. Chem. Ind.*, 1911, 30, 62)

	Sample 1	Sample 2	Sample 3	Sample 4
1. Direct alkaline (basic lead) polarisation	48.8		48.6	47.0
2. Direct neutral polarisation	49.2	52.0	49.0	47.6
3. Direct acid (Andrlik) polarisation	50.4	53.25	50.0	48.4
4. Direct acid (SO <sub>2</sub> ) polarisation (Pellet)	50.3	53.3		48.3
5. Invertase inversion polarisation	-14.6	-16.0	-15.0	-14.4
6. Acid inversion polarisation	-13.2	-14.6	-13.6	-13.8
7. Clerget value by invertase	48.5	51.7	48.6	47.1
8. Clerget value by acid, using alkaline direct polarisation	47.0		47.1	46.1
9. Clerget value by acid, using neutral direct polarisation	47.3	50.5	47.4	46.5
10. Clerget value by acid, using acid (Andrlik) direct polarisation	48.2	51.4	48.2	47.1
11. Clerget value by acid, using acid (SO <sub>2</sub> ) direct polarisation (Pellet)	48.1	51.4		47.0

From these tables it is seen that with *beet* molasses the results obtained by the ordinary Herzfeld process using the alkaline direct polarisation are *low* as compared with the practically identical values obtained by using invertase or the direct acid polarisation. In the case of *cane* molasses the reverse is true, the results obtained by the ordinary method being *high*. The difference is probably due to the different character of the disturbing factor in the two cases; in beet molasses it is the presence of amides and amino-acids, and in cane molasses it is the reducing sugars.

According to Cross and Taggart (*Louisiana State University Agriculture Bulletin*, No. 135, December, 1912) the retarding action of betaine and urea on the inversion of sucrose by hydrochloric acid is only slight at temperatures of 20 to 28°, and therefore analytical methods based on this supposed retarding influence, such as the Andrlik process, are not satisfactory. According to Pellet (*loc. cit.*) in the Andrlik method the polarisation of the acid solution should be completed in less than 7 minutes if change of the sucrose is to be avoided.

There is perhaps still some doubt whether the values obtained by the Pellet and Andrlik processes actually represent the true sucrose present; the agreement between the results obtained by the two methods may be fortuitous and it is worthy of note that the original Pellet method, in which a smaller proportion of sulphur dioxide was used, gave slightly different results and has been modified by increasing the amount of sulphur dioxide present. On the other

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hand, it is a confirmation of some value that the invertase method gives practically identical results (Ogilvie).

Fribourg (*Int. Sugar J.*, 1912, 14, 702) has made an exhaustive study of the various methods of estimating sucrose in cane molasses; he shows that the Pellet, Andriik, and invertase methods of inversion give results which agree well amongst themselves and also with that obtained gravimetrically by copper reduction before and after inversion.

**Inversion of Sucrose by Citric Acid.**—In estimating sucrose in plant extracts it is inadvisable to invert with hydrochloric acid at 70° under Herzfeld conditions as a certain proportion of any maltose present undergoes hydrolysis to dextrose.<sup>1</sup> In such cases a weaker acid such as citric acid can be applied but error may arise unless special precautions are taken. Davis and Daish (*J. Agric. Sci.*, 1913, 5, 473) show that in a series of experiments carried out on mangold leaf extracts from which tannins, amino-acids, etc., had been as far as possible removed by basic lead acetate in the usual way, the amount of sucrose found by inverting with 2% citric acid was very small as compared with the amount found by means of invertase. It was ultimately discovered that the cause of the difference was the presence in solution of a relatively large proportion of sodium acetate, which almost entirely inhibits the invertive action of 2% citric acid. The sodium acetate was produced owing to the necessity, in the cases dealt with, of using relatively large quantities of basic lead acetate to remove the tannins, etc., present in the extracts analysed; on subsequently adding sodium carbonate to precipitate the slight excess of lead present in the filtered solution the whole of the acetic acid originally present in the basic lead acetate was converted into sodium acetate. It is an interesting fact that although sodium acetate, when present to the extent of about 1 to 2%, almost completely inhibits inversion of sucrose by boiling 2% citric acid, it does not in the least interfere with the action of invertase (autolysed yeast); this shows the advantage of using invertase in many cases as a means of checking the results obtained by acid hydrolysis. According to Davis, however, if citric acid of 10% concentration

<sup>1</sup> It is generally stated that maltose is not hydrolysed at all by hydrochloric acid under Herzfeld conditions, and Kluver (*Biochemische Suikerbepalingen*, 1914, page 223) considered that Davis and Daish were in error in considering this statement to be incorrect; Davis has however since shown (*J. Agric. Sci.*, 1914, 6, 413) that under Herzfeld conditions the hydrolysis of maltose is quite appreciable and sufficient to interfere with the accuracy of the sucrose estimation.

is used complete hydrolysis of the sucrose is effected, even when very large quantities of basic lead acetate have been employed in the preliminary purification of the solutions dealt with. The method of carrying out the hydrolysis by 10% citric acid is as follows: 50 c.c. of the solution to be analysed (which has had the excess of lead removed by means of sodium carbonate) is treated with a few drops of concentrated sulphuric acid until it just shows the faintest indication of pink to methyl-orange. Solid citric acid is then added so as to make a 10% solution (5 grm. solid citric acid to 50 c.c. of solution) and the mixture heated to boiling; it is kept actively boiling over a small flame for 10 minutes, under a reflux condenser, and the solution is then cooled to the ordinary temperature and exactly neutralised (using phenolphthaleïn as indicator) by adding a 10 or 15% solution of sodium hypoxide. It is diluted to 100 c.c. at 20° and is then ready for the dolarisation or for measuring the reducing power by means of Fehling's solution.

**Incomplete Inversion Caused by the Use of Large Quantities of Basic Lead Acetate or by the Presence of Salts of Organic Acids.**—In the ordinary analysis of sugar works materials and products the quantity of basic lead acetate used is generally relatively small, and as much as 5 c.c. of the lead solution can be present in excess without interfering with the completeness of the inversion under Herzfeld conditions (see Pellet, *Dosage du Sucre*, page 39). On the other hand, in working with vegetable extracts, when much larger quantities of basic acetate have to be used, the proportion of lead which remains unprecipitated in the solution may be so great as to lead to incomplete inversion if only the usual quantity of hydrochloric acid be employed in the Herzfeld method; even if this lead be precipitated by sodium carbonate or sulphate, sodium acetate is formed, which inhibits more or less completely the invertive action of the hydrochloric acid just as it does that of citric acid (see page 386). In such cases serious error in the analysis may arise. Care should be taken in such cases to make the solution very faintly acid to methyl-orange by the addition of sulphuric or hydrochloric acid before adding the quantity of acid (5 c.c.) required under Herzfeld conditions. Duplicate experiments can also be profitably made in which the period of heating is prolonged to 10 or 15 minutes; Pellet has shown that under Herzfeld conditions the time of heating can be prolonged to 20 minutes (at 70°) without affecting the accuracy of the results;

on the other hand, it is not desirable to increase the quantity of hydrochloric acid used to 10 c.c., as the extra proportion of acid used sensibly modifies the rotatory power of the invert sugar present.

When working with plant material or plant extracts, in the purification of which relatively large quantities of basic lead acetate have been employed, it is always advisable to carry out a control estimation of the sucrose by means of invertase, inversion by which is complete even when sodium acetate is present in considerable quantity. It must be noted that when maltose is present, there is risk of bringing about considerable hydrolysis of this sugar to dextrose, if the time of heating be prolonged under the Herzfeld conditions by 5 minutes. Even with 5 minutes' heating marked hydrolysis occurs, so that when maltose (or similar glucosides) are present it is advisable to use citric acid for the inversion under the conditions already defined (page 386).

**Polarimetric Estimation of Sucrose in Presence of Raffinose.**—Raffinose is a normal constituent of the sugar beet, which, according to Strohmer (*Deut. Zuckerind.*, 1910, **35**, 543) contains, on the average, 0.02% of this sugar. In the process of extracting sugar from the beet, the raffinose accumulates in the residual molasses, and whilst first product raw beet sugar is generally free from raffinose, beet molasses and sugar obtained by the de-saccharification of beet molasses (so-called "process" sugars) are liable to contain it. Since raffinose is much more dextrorotatory than sucrose, the presence of the former makes the sucrose-content of the material appear to be considerably greater than is actually the case. Sucrose is generally determined in raffinose-containing samples by the same method of polarisation before and after inversion with hydrochloric acid as is used for determining it in presence of reducing sugars (see page 377). The percentages of sucrose,  $S$ , and of raffinose,  $R$ , are calculated by the formulæ:

$$S = \frac{0.5124P - (-I)}{0.839}; \quad R = \frac{P - S}{1.852}$$

in which  $P$  is the direct polarisation and  $I$  is the polarisation of the inverted solution referred to normal concentration (*i. e.*, twice the actual reading). The readings must be taken at 20° in order that these formulæ shall apply. At any other temperature ( $T$ ) the following formula should be used.

$$S = \frac{P(0.4724 + 0.002T) - (-I)}{0.899 - 0.003T}$$

Having calculated  $S$ , then  $R = \frac{P - S}{1.852}$ .

The use of this method, however, in the case of vegetable material and beet molasses may return as raffinose numbers which are far from representing the true proportion of this substance present. This is due to the presence of other optically active substances, such as the glutamic and aspartic acids, the specific rotation of which undergoes a considerable change during the process of inversion. Pellet (*Dosage du Sucre*, page 63) gives an example of a crude sugar, showing a direct polarisation of 91.10 by the ordinary method (in presence of basic lead acetate) which, when examined according to the Herzfeld method, appeared to contain 0.5% of raffinose; when, however, the direct polarisation was taken in presence of acid, the value found was 91.55 and the conclusion drawn was that no raffinose was actually present. Other sources of error in the estimation of raffinose may be incomplete inversion of the sucrose owing to the presence of basic lead or sodium acetate (page 387), the presence of pentoses, etc.

From the above considerations it is clear that the estimation of raffinose by the ordinary method, now in general use, gives results upon which in the majority of cases little reliance can be placed.

Hudson and Harding (*J. Amer. Chem. Soc.*, 1915, **37**, 2193) have devised a biochemical method for estimating raffinose in sugar mixtures and plant materials which depends on the transformation of raffinose into melibiose and lævulose by invertase (prepared from top fermentation yeast) followed by the transformation of the melibiose into galactose and dextrose by *melibiase* (prepared from bottom fermentation yeast). The change in rotation due to the hydrolysis of melibiose (from  $[\alpha]_D = +143^\circ$  to  $+70.4^\circ$ ) serves to measure the amount of raffinose originally present. For details of the process reference must be made to the original paper.

**Mutarotation.**—Considerable confusion has in the past been introduced into optical saccharimetry owing to the changes in rotatory power shown by freshly dissolved sugars on keeping, a phenomenon known as "mutarotation." This change has been shown by E. F. Armstrong and T. M. Lowry (*Trans. Chem. Soc.*, 1903, **83**, 1305,

1314) to be due to the mutual interconversion in solution of two isomerides of the sugar. Most sugars exist in solution as a mixture of two forms in equilibrium. Thus in the case of dextrose the anhydrous solid is the  $\alpha$ -modification of high rotatory power, which persists as such in the freshly made solution but slowly passes over in part into the  $\beta$ -form of low rotatory power. The change is much accelerated by impurities, particularly those of an alkaline nature. The addition of a trace of alkali to a freshly-made solution of dextrose causes a sudden fall in the rotation—this has been made use of to identify the various forms of this sugar.

All products, such as honeys, syrups, etc., which contain dextrose or other reducing sugars in the crystalline form or in supersaturated solution, exhibit the phenomenon of mutarotation. The constant rotation only should be employed in the Clerget formula, and to obtain this the solutions prepared for direct polarisation should be allowed to stand over night before making the reading. In case it is desired to make the direct reading immediately the mutarotation may be destroyed by heating the neutral solution to boiling for a few minutes or by adding a few drops of strong ammonia before completing the volume.

## METHOD OF ANALYSIS DEPENDING ON REFRACTIVE INDEX

This method may be used in the same manner as the sp. gr. method, over which it has advantages in speed and ease of manipulation. The most recent experience shows that the refractometric method gives trustworthy results even with highly concentrated and very crude syrups. When using the Pulfrich instrument, as done by Stolle (*Zeitsch. Ver. deut. Zuckerind.*, 1901, 335, 469), 5 c.c. of solution are necessary; with the Abbé refractometer a few drops suffice. Working with the latter instrument, Tolman and Smith (*J. Amer. Chem. Soc.*, 1906, 28, 1476) find that for the same concentration the index of refraction is practically the same for sucrose, maltose, lactose, dextrose, lævulose, and commercial glucose, but is somewhat higher for dextrin. The following table gives the refractive index for sucrose solutions of varying strengths at 20°. The temperature correction is practically the same as that for sp. gr.

Sucrose, %	Index of refraction at 20°	Sucrose, %	Index of refraction at 20°	Sucrose, %	Index of refraction at 20°
1	1.3343	31	1.3828	61	1.4442
2	1.3357	32	1.3847	62	1.4465
3	1.3372	33	1.3865	63	1.4488
4	1.3387	34	1.3883	64	1.4511
5	1.3402	35	1.3902	65	1.4534
6	1.3417	36	1.3921	66	1.4557
7	1.3432	37	1.3940	67	1.4581
8	1.3447	38	1.3959	68	1.4605
9	1.3462	39	1.3978	69	1.4629
10	1.3477	40	1.3997	70	1.4653
11	1.3492	41	1.4017	71	1.4677
12	1.3508	42	1.4036	72	1.4701
13	1.3524	43	1.4056	73	1.4726
14	1.3539	44	1.4076	74	1.4751
15	1.3555	45	1.4096	75	1.4776
16	1.3572	46	1.4117	76	1.4801
17	1.3588	47	1.4137	77	1.4826
18	1.3604	48	1.4158	78	1.4851
19	1.3621	49	1.4179	79	1.4877
20	1.3637	50	1.4200	80	1.4903
21	1.3654	51	1.4221	81	1.4929
22	1.3671	52	1.4242	82	1.4955
23	1.3688	53	1.4263	83	1.4981
24	1.3705	54	1.4284	84	1.5007
25	1.3722	55	1.4306	85	1.5034
26	1.3739	56	1.4328	86	1.5061
27	1.3756	57	1.4351	87	1.5088
28	1.3774	58	1.4373	88	1.5115
29	1.3792	59	1.4396	89	1.5142
30	1.3810	60	1.4419	90	1.5170

For recent tables of refractometric values, reference may be made to Main (*Int. Sugar J.*, 1907, 9, 481) and Schönrock (*Zeitsch. Ver. deut. Zuckerind.*, 1911 61, [2], 421).

## METHODS OF ANALYSIS DEPENDING ON REDUCING POWER

**Reactions of the Sugars as Reducing Agents.**—Most of the carbohydrates, with the notable exception of sucrose and raffinose, possess marked activity as reducing agents.

In hot alkaline solution, the hexoses reduce picric acid to picramic acid, indigotin to indigo white, and change ferricyanides to ferrocyanides. Bismuth, mercury, silver, platinum, and gold salts are reduced to metal, and ferric and cupric salts to ferrous and cuprous compounds respectively.

The reducing properties of sugars are best manifested and measured by their reaction towards alkaline solutions of cupric and mercuric salts, and the processes in which these are employed require to be described in detail.

In the first place, the well-established standard processes will be dealt with, followed by a brief résumé of the more recent modifications of these methods, some of which perhaps still require confirmation by other workers before being universally adopted. They have been in many cases devised to solve the difficulties presented by special problems.

**Reaction of Sugars with Cupric Salts in Alkaline Solution.—**

If a solution of cupric sulphate be mixed with a sufficient quantity of a saccharine liquid, no precipitate of copper hydroxide is produced on addition of sodium or potassium hydroxide. The liquid becomes deep blue, but remains clear. On raising the fluid to boiling point no visible change occurs if the liquid contains sucrose only, but, if any form of hexose is present, a yellow precipitate of cuprous hydroxide is produced, which quickly turns to cuprous oxide and becomes an orange-red. If the hexose is present in excess the blue of the solution entirely disappears. Instead of relying on a saccharine substance for the prevention of the precipitation of the cupric hydrate by the alkali it is far better to employ a tartrate, as in Fehling's solution.

The reducing action of certain forms of sugar on alkaline solutions of copper has been applied by different chemists in many ways, the precipitated cuprous oxide being weighed as such by several, by others converted into metallic copper or cupric oxide, and by others redissolved and estimated volumetrically. Some operators make the original process a volumetric one. The great majority of these modified processes are merely of historical interest and require no detailed description.

**Fehling's Solution.**—The alkaline solution of copper most commonly employed for the determination of sugars is that known as Fehling's which is essentially a solution of copper sodium tartrate containing a considerable quantity of sodium hydroxide.<sup>1</sup> It is best prepared in the following manner, known as Soxhlet's modification: 34.639 grms. of pure crystallised copper sulphate (free from iron and moisture) are dissolved in distilled water, and the solution diluted to 500 c.c. 50 grms. of sodium hydroxide of good quality (not less than 97% NaOH) and 173 grms. of recrystallised potassium sodium tartrate are dissolved in about 400 c.c. of water and the solu-

<sup>1</sup> For the nature of the salts existing in Fehling's solution see Masson and Steele, *Trans. Chem. Soc.*, 1899, 75, 725, and Bullheimer and Seitz, *Ber.*, 1900, 33, 807.

tion diluted to 500 c.c. Fehling's solution is prepared by carefully adding the copper sulphate solution to an equal measure of the alkaline tartrate solution. It may be kept ready mixed, but should in that case be carefully protected from air and light, as it is apt to undergo changes which render its indications unreliable. Before using it is desirable to ascertain its condition, by diluting a quantity with an equal volume of water and heating the liquid to boiling for a few minutes. It ought to remain perfectly clear. It is preferable to keep the copper and tartrate solutions separate and mix them in equal measures at frequent intervals.

Numerous modifications of Fehling's solution have been recommended and used by different chemists from time to time, the variations for the most part being in respect of the concentration and kind of caustic alkali employed. Further, the procedures adopted in using the various modifications of Fehling's solution differ considerably one from another. Since the amount of copper reduced by a given weight of a reducing sugar is affected by various factors, such as the amount of free alkali, the period and method of heating, the concentration of the reaction mixture, and the presence of excess of copper, it is evident that in making a quantitative determination by any given method, the particular modification of Fehling's solution and the procedure for using it must be strictly adhered to, in order that the tables and data given for the method in question may be applicable.

For the *detection of reducing sugar* in clear, colourless solution, all that is necessary is to neutralise any free acid and heat the liquid to boiling with a little Fehling's solution. If a yellow or orange-red turbidity or precipitate of cuprous oxide be produced, a reducing sugar, or some substance giving a similar reaction, is present. The hexoses and maltose reduce the copper solution with facility, but sucrose gives no reaction until after inversion.

If the liquid is much coloured it is difficult or impossible to recognise properly the reaction with Fehling's solution. Coloration of the liquid is still more objectionable if the sugar is to be estimated by the volumetric process. In such cases the sugar solution must be clarified by one of the methods employed for the preparation of a solution for the polarimeter (see page 374), but if lead has been employed it must be *completely* removed from the solution or the results of the test will be worthless. According to the A. O. A. C.,

the clarification of sugar solutions that are to be used for determination of invert sugar must be effected with neutral lead acetate solution and the excess of lead removed with potassium oxalate before making the determination.

Fehling's solution may be used volumetrically or gravimetrically. Both methods are capable of giving useful approximate results, but if any high degree of accuracy be sought it is essential that there should be strict adherence to certain conditions of manipulation.

**General Methods.**—Certain sources of error in estimating sugars by gravimetric and volumetric methods are dealt with in a paper by Davis and Daish (*J. Agric. Sci.*, 1913, 5, 437). In the gravimetric method, in which the precipitate of cuprous oxide obtained is collected on asbestos, the necessity of previously digesting the asbestos with boiling 20% sodium hydroxide solution and subsequently thoroughly washing it with water is emphasized; unless the asbestos is treated in this way considerable loss of weight may occur owing to the action of the hot Fehling's solution on impurities present in the asbestos. It is probably best and simplest to collect the cuprous oxide precipitate in a Gooch crucible containing a layer  $\frac{1}{16}$  to  $\frac{1}{8}$  in. thick of the purified asbestos and, after a thorough washing with boiling water, to wash it with a little alcohol and ether and dry it in a steam-oven. The Gooch crucible is then placed in an ordinary No. 1 Berlin crucible (which serves to shield it from direct contact with the flame) and is heated strongly over a  $\frac{1}{2}$ -in. Teclu or Fletcher Argand gas flame for  $\frac{1}{2}$  hour; it is then allowed to cool in the desiccator at least 1 hour, weighed and again heated for another 30 minutes. The weight is generally practically constant after the first heating, the increase of weight in the second heating seldom exceeding 0.0005 gm. If the Gooch crucible be shielded from direct contact with the flame in the way indicated there is never any difficulty in obtaining accurate results for the weight of cupric oxide, and the process is far more simple and rapid than collecting the cuprous oxide in a Soxhlet tube and reducing to copper in a stream of hydrogen in the manner generally advocated. The same crucible and asbestos can be used over and over again for 10 to 20 successive charges without dissolving away the cupric oxide; the fresh charge of cuprous oxide is collected on the top of the previous charge of cupric oxide. It is only necessary to ensure that this is constant in weight. Elion (*Zeitsch. angew. Chem.*, 1890, 325) and others have stated that con-

version to cupric oxide gives unreliable and discordant results, but this is not true when the cupric oxide is shielded from the reducing gases of the flame. Under the conditions given the ratio of  $\frac{2\text{CuO}}{\text{Cu}_2\text{O}}$  is found to range from 1.111 to 1.112, the theoretical ratio (Cu = 63.57) being 1.112. H. Pellet recommends igniting the cuprous oxide precipitate by heating it *at not too high a temperature* in a muffle furnace. A blowpipe should never be used, even when the Gooch crucible is shielded by an outer crucible, as low results are then obtained, probably owing to the slight dissociation of cupric oxide which occurs at very high temperatures: the ratio  $\frac{2\text{CuO}}{\text{Cu}_2\text{O}}$  when the blowpipe is used ranges from 1.105 to 1.109 instead of having practically the theoretical value 1.112 (Davis and Daish, *loc. cit.*).

The recommendation is frequently made to weigh the cuprous oxide, as such, after drying at 100°. Whilst this course is quite safe in the case of pure sugars it involves considerable error when dealing with impure solutions containing organic substances such as are obtained when working with plant or animal extracts, even when these have been partially purified by treatment with basic lead acetate, etc.; the same is true of the solutions obtained on inverting or hydrolysing starch or sugars by enzyme preparations, such as diastase, maltase or invertase, or after fermenting sugars by yeasts, even though alumina cream is subsequently used to clear the solutions. In all such cases, the cuprous oxide invariably contains organic matter, which burns away during ignition, so that the ratio  $\frac{2\text{CuO}}{\text{Cu}_2\text{O}}$  is thereby diminished; this ratio varies from 1.060 to 1.105, according to the nature of the solution and of the enzyme preparation employed. It is probable that in dealing with yeasts, invertase, etc., the cuprous oxide precipitate contains traces of copper compounds of amino-acids, proteins, etc., as well as colloidal matter carried down by absorption. In such cases the cupric oxide weighed would be slightly higher than that actually due to reduction only; but numerous experiments indicate that this error is relatively small and not likely to interfere with the results obtained. An alternative method of procedure in such cases is to estimate the actual copper present in the cuprous oxide precipitate by one of the standard volumetric methods (see below, page 416).

The method which depends on using ferric sulphate to dissolve the cuprous oxide and subsequently titrating back with permanganate (see page 416) is criticised by Davis and Daish (*loc. cit.*) who came to the conclusion that it cannot be regarded as one of the most accurate methods for the purpose. This method has, however, been widely used in recent years. In biochemical work it has been extensively employed since Bertrand advocated its use (*Bull. Soc. Chim.*, 1906, [iii], 35, 1285) and gave tables for dextrose, invert sugar, maltose and lactose. Davis and Daish state that in their hands this method gave values from 1 to 1.5% in error for pure dextrose and maltose and point out that the constants given by Bertrand for his dextrose ( $[\alpha]_D = 52.0^\circ$ ) and maltose ( $137.4^\circ$ ) are not those of sugars of the highest degree of purity. In preparing the solution of invert sugar on which his tables are based, Bertrand hydrolysed sucrose by heating with 2% hydrochloric acid for 10 to 15 minutes at  $100^\circ$ ; such treatment invariably causes slight destruction of l  vulose, and Davis and Daish in consequence found results by using Bertrand's tables which were 3 to 5% low. That decomposition of invert sugar occurs with dilute hydrochloric acid at temperatures above  $70^\circ$  has been generally recognised since the work of Herzfeld (*Zeitsch. Ver. deut. Zuckerind.*, 1888, 38, 699). The volumetric method using permanganate is, too, not so advantageous as the method of Ling, Rendle and Jones (see below, page 419) either on the ground of rapidity (for which it is generally preferred to gravimetric methods) or of accuracy. It is to be regarded only as a fairly rapid approximate method which may prove useful when no high degree of accuracy is required.

**Gravimetric Methods for Reducing Sugars.**—Probably the most accurate method of estimating the reducing sugars (dextrose, l  vulose, invert sugar and maltose) is to work under the conditions laid down by Brown, Morris and Millar (*Trans. Chem. Soc.*, 1897, 71, 94) employing the tables they have given for these sugars. Davis and Daish using highly purified specimens of the sugars verified these tables and found a quite satisfactory agreement. The probable error in the copper oxide weighed under these conditions is not likely to be more than 1 mg., which, when 0.20 to 0.40 gm. is actually obtained, gives an error well within 0.5%. It is certainly preferable in the majority of cases to work under the well-defined conditions laid down by Brown,

Morris and Millar than to use Allihn's method, in which the size of the beaker and the kind and height of the flame undoubtedly influence the results; the shortness of the time of heating is, too, a disadvantage, as any slight differences thus exercise proportionately greater effect. The tables given by Wein for maltose, which have been generally used, were shown by Brown, Morris and Millar in sin 1897 to give results 5% low; Ling and Baker (*Trans. Chem. Soc.*, 1897, 71, 509) confirmed this.

**Brown, Morris and Millar's Method.**—This is a modification of the gravimetric process suggested by O'Sullivan (*J. Chem. Soc.*, 1876, II, 130), the essential point being that regular heating is ensured by immersing the beaker during the reduction in boiling water; the time of heating is 12 minutes. The solutions used are as follows:

- |                                    |           |
|------------------------------------|-----------|
| (A) Recrystallised copper sulphate | 34.6 gm.  |
| Water to                           | 500 c.c.  |
| (B) Rochelle salt                  | 173.0 gm. |
| Anhydrous sodium hydroxide         | 65.0 gm.  |
| Water to                           | 500 c.c.  |

As the oxidising power of the solution is very sensitive to small changes in the amount of alkali present great care must be taken to ensure accuracy in the weight of sodium hydroxide. This is best done by preparing a somewhat concentrated solution of sodium hydroxide (free from carbonate), ascertaining the strength by means of the density, and then diluting so that a known volume (300 c.c., for example) represents exactly 65.0 gm. It is convenient to prepare large volumes (7 to 10 litres) of each of the solutions *A* and *B* and to store these in large glass bottles (Welsbach bottles) so arranged as to feed two 25 c.c. automatic pipettes. Access of carbon dioxide of the air to solution *B* should be guarded against by a U-tube containing soda-lime.

25 c.c. of each solution *A* and *B* are introduced into a beaker, the surface area of which<sup>1</sup> is 44 sq. cm.; the beaker is covered with a clock glass and immersed in a bath of *boiling* water until the temperature

<sup>1</sup> A Jena beaker flask of 250 c.c. can be conveniently used here; such vessels, with a top diameter  $2\frac{1}{4}$  in., bottom diameter  $2\frac{1}{8}$  in., give accurately the results contained in Brown, Morris and Millar's tables (Davis and Daish, *loc. cit.*), and are far more convenient in manipulation than ordinary beakers. Pellet, also (*Bull. Assoc. Chim. Sucr.*, 1913, 198), has independently suggested the use of beaker flasks for measurements of the reducing power of sugars.

of the solution is the same as that of the bath. This is usually the case in 6 minutes. The sugar solution is now added and the total volume diluted if necessary to 100 c.c.; it is best if possible to have the sugar solution of such a concentration that 50 c.c. can be taken directly for the estimation. If the sugar solution is considerably

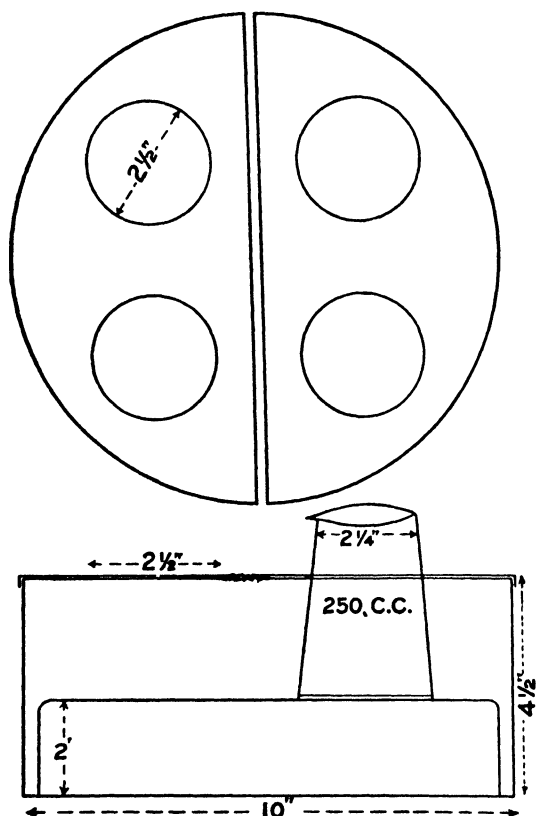


FIG. 59.

more concentrated, 25 c.c. (or 30 c.c.) are taken and a corresponding quantity of boiling water (25 c.c. or 20 c.c.) added. The beaker is then immediately covered with the clock glass and heated exactly 12 minutes in boiling water; the precipitated cuprous oxide is then filtered off as rapidly as possible in a Soxhlet tube or preferably a

Gooch crucible, thoroughly washed with about 400 c.c. of boiling water, dried and weighed, either as cupric oxide (ignition as above) or after reduction to metallic copper.

The amount of reducing sugar taken for an estimation should give a weight of cupric oxide lying within the limits 0.15 to 0.40 gm. The table below gives the relationship existing between the weight of copper (or of CuO) and the various sugars.

A convenient heating bath is shown in Fig. 59 for use with the beaker flasks employed in the reduction (Davis and Daish, *loc. cit.*). It consists of a 10 in. enamelled iron saucepan, 4.5 in. deep, into which a false bottom of copper plate is placed so as to afford a convenient support for the beaker flasks. The cover of the bath is made of copper and consists of two halves each perforated with two

TABLE—REDUCING POWER OF DEXTROSE, LÆVULOSE AND INVERT SUGAR UNDER BROWN, MORRIS AND MILLAR'S CONDITIONS

Sugar, mg.	Dextrose			Lævulose			Invert sugar		
	Cu, gm.	CuO, gm.	CuO corresponding to 1 gm. dextrose	Cu, gm.	CuO, gm.	CuO corresponding to 1 gm. lævulose	Cu, gm.	CuO, gm.	CuO corresponding to 1 gm. invert sugar
50	0.1030	0.1280	2.578	0.0923	0.1155	2.340	0.0975	0.1221	2.442
55	0.1134	0.1422	2.585	0.1027	0.1287	2.341	0.1076	0.1349	2.453
60	0.1238	0.1552	2.587	0.1122	0.1407	2.345	0.1176	0.1474	2.457
65	0.1342	0.1682	2.589	0.1216	0.1524	2.346	0.1275	0.1598	2.459
70	0.1443	0.1809	2.585	0.1312	0.1645	2.350	0.1373	0.1721	2.459
75	0.1543	0.1935	2.580	0.1405	0.1761	2.349	0.1468	0.1840	2.454
80	0.1644	0.2061	2.577	0.1500	0.1881	2.351	0.1566	0.1963	2.454
85	0.1740	0.2187	2.572	0.1590	0.1993	2.345	0.1662	0.2084	2.451
90	0.1834	0.2299	2.555	0.1686	0.2114	2.349	0.1755	0.2200	2.445
95	0.1930	0.2420	2.547	0.1774	0.2224	2.341	0.1848	0.2317	2.439
100	0.2027	0.2538	2.538	0.1862	0.2331	2.331	0.1941	0.2430	2.430
105	0.2123	0.2662	2.535	0.1952	0.2447	2.331	0.2034	0.2550	2.429
110	0.2218	0.2781	2.528	0.2040	0.2558	2.325	0.2128	0.2668	2.425
115	0.2313	0.2900	2.522	0.2129	0.2669	2.321	0.2220	0.2783	2.420
120	0.2404	0.3014	2.512	0.2215	0.2777	2.314	0.2311	0.2898	2.415
125	0.2496	0.3130	2.504	0.2303	0.2887	2.310	0.2400	0.3009	2.407
130	0.2585	0.3241	2.493	0.2390	0.2997	2.305	0.2489	0.3121	2.400
135	0.2675	0.3354	2.484	0.2477	0.3106	2.300	0.2578	0.3232	2.394
140	0.2762	0.3463	2.473	0.2559	0.3209	2.292	0.2663	0.3339	2.385
145	0.2850	0.3573	2.464	0.2641	0.3311	2.284	0.2750	0.3448	2.378
150	0.2934	0.3673	2.448	0.2723	0.3409	2.273	0.2832	0.3546	2.364
155	0.3020	0.3787	2.443	0.2805	0.3517	2.269	0.2915	0.3655	2.358
160	0.3103	0.3891	2.432	0.2889	0.3622	2.264	0.3002	0.3764	2.352
165	0.3187	0.3996	2.422	0.2972	0.3726	2.258	0.3086	0.3869	2.345
170	0.3268	0.4098	2.410	0.3053	0.3828	2.252	0.3167	0.3971	2.336
175	0.3350	0.4200	2.400	0.3134	0.3930	2.245	0.3251	0.4076	2.329
180	0.3431	0.4302	2.390	0.3216	0.4032	2.240	0.3331	0.4177	2.320
185	0.3508	0.4399	2.377	0.3297	0.4134	2.234	0.3410	0.4276	2.311
190	0.3590	0.4501	2.369	0.3377	0.4234	2.228	0.3490	0.4376	2.303
195	0.3668	0.4599	2.358	0.3457	0.4335	2.223	0.3570	0.4476	2.295
200	0.3745	0.4689	2.344	0.3530	0.4431	2.216	0.3650	0.4570	2.285
205	0.3822	0.4792	2.338	0.3616	0.4534	2.211	0.3726	0.4672	2.279

TABLE.—REDUCING POWER OF MALTOSE UNDER BROWN, MORRIS AND MILLAR'S CONDITIONS

Maltose, mg.	Cu weighed, grm.	CuO weighed, grm.	CuO corresponding to 1 grm. maltose	Maltose, mg.	Cu weighed, grm.	CuO weighed, grm.	CuO corresponding to 1 grm. maltose
70	0.0772	0.0966	1.380	190	0.2072	0.2593	1.371
75	0.0826	0.1034	1.380	195	0.2126	0.2661	1.370
80	0.0880	0.1102	1.379	200	0.2180	0.2729	1.370
85	0.0934	0.1169	1.379	205	0.2234	0.2797	1.370
90	0.0988	0.1237	1.378	210	0.2288	0.2865	1.369
95	0.1042	0.1305	1.378	215	0.2342	0.2933	1.369
100	0.1097	0.1373	1.378	220	0.2397	0.3000	1.3685
105	0.1151	0.1441	1.377	225	0.2451	0.3068	1.368
110	0.1205	0.1509	1.377	230	0.2505	0.3136	1.368
115	0.1259	0.1576	1.3765	235	0.2559	0.3203	1.367
120	0.1313	0.1644	1.376	240	0.2613	0.3272	1.367
125	0.1367	0.1712	1.376	245	0.2667	0.3340	1.3665
130	0.1422	0.1779	1.375	250	0.2722	0.3407	1.366
135	0.1476	0.1848	1.375	255	0.2776	0.3475	1.366
140	0.1530	0.1916	1.375	260	0.2830	0.3543	1.365
145	0.1584	0.1983	1.374	265	0.2884	0.3610	1.365
150	0.1634	0.2051	1.374	270	0.2938	0.3678	1.365
155	0.1692	0.2119	1.373	275	0.2992	0.3747	1.364
160	0.1747	0.2186	1.373	280	0.3047	0.3814	1.364
165	0.1801	0.2254	1.373	285	0.3101	0.3882	1.3635
170	0.1855	0.2323	1.372	290	0.3155	0.3950	1.363
175	0.1909	0.2390	1.372	295	0.3209	0.4017	1.363
180	0.1963	0.2458	1.3715	300	0.3264	0.4085	1.362
185	0.2017	0.2526	1.371	305	0.3318	0.4154	1.362

2.5 in. holes, the edge of the plate being turned down so as to fit over the bath. Each half of the cover can be lifted off separately so as to admit the beaker flask containing the Fehling solution; with this arrangement several beaker flasks can be heated simultaneously.

The most carefully prepared Fehling's solution usually gives a very slight precipitate of cuprous oxide on heating, owing to spontaneous reduction; this value should be determined for every fresh batch of solution and be allowed for in the final result of each determination. It usually amounts to 0.002 to 0.003 grm. per 50 c.c. of Fehling's solution. W. A. Davis has observed that when the two solutions *A* and *B* after being prepared are separately stored in stoppered bottles for a week or fortnight before use a considerable quantity of sediment separates from each; when the solutions are filtered and then tested in the usual way, in a "blank" experiment, the amount of self-reduction is very small, usually 0.0000 to 0.0010 grm. per 50 c.c. of solution. The "self-reduction" thus rapidly diminishes immediately after the solutions are made up, so that it is always advisable to store the solutions for at least a fortnight before use.

**Official A. O. A. C. Gravimetric Method.**—With the following method, the tables compiled by Munson and Walker (*J. Amer. Chem. Soc.*, 1906, **28**, 663; 1907, **29**, 541), given below, are to be used.

### 1. Preparation of Solutions and Asbestos

(a) **Solutions.**—Use Soxhlet's modification of Fehling's solution (see page 392).

(b) **Asbestos.**—Prepare the asbestos, which should be the amphi-hole variety, by first digesting it with 1:3 hydrochloric acid for 2 or 3 days. Wash free from acid and digest for a similar period with sodium hydroxide solution, after which treat for a few hours with hot alkaline copper tartrate solution of the strength employed in sugar determinations. Then wash the asbestos free from alkali, finally digest with nitric acid for several hours and, after washing free from acid, shake with water for use. In preparing the Gooch crucible load it with a film of asbestos  $\frac{1}{4}$  in. thick, wash this thoroughly with water to remove fine particles of asbestos; finally wash with alcohol and ether, dry for 30 minutes at 100°, cool in a desiccator and weigh. It is best to dissolve the cuprous oxide with nitric acid each time after weighing and use the same felts over and over again, as they improve with use.

### 2. Determination

Transfer 25 c.c. each of the copper and alkaline tartrate solutions to a 400 c.c. beaker and add 50 c.c. of reducing sugar solution, or, if a smaller volume of sugar solution be used, add water to make the final volume 100 c.c. Heat the beaker upon an asbestos gauze over a Bunsen burner, so regulate the flame that boiling begins in 4 minutes, and continue the boiling for exactly 2 minutes. Keep the beaker covered with a watch-glass throughout the entire time of heating. Without diluting, filter the cuprous oxide at once on an asbestos felt in a porcelain Gooch crucible, using suction. Wash the cuprous oxide thoroughly with water at a temperature of about 60°, then with 10 c.c. of alcohol and finally with 10 c.c. of ether. Dry for 30 minutes in a water-oven at 100°, cool in a desiccator and *weigh as cuprous oxide*.

N. B. The number of milligrams of copper reduced by a given amount of reducing sugar differs when sucrose is present and when it

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TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE,  
INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRM. AND  
2 GRM. TOTAL SUGAR), AND MALTOSE (ANHYDROUS AND  
CRYSTALLISED)

(For Lactose Figures see pages 457 to 460)  
(Expressed in milligrams)

Cuprous oxide (Cu <sub>2</sub> O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and sucrose		Maltose		Cuprous oxide (Cu <sub>2</sub> O)
				0.4 grm. total sugar	2 grm. total sugar	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> + H <sub>2</sub> O	
10	8.9	4.0	4.5	1.6	....	5.9	6.2	10
11	9.8	4.5	5.0	2.1	....	6.7	7.0	11
12	10.7	4.9	5.4	2.5	....	7.5	7.9	12
13	11.5	5.3	5.8	3.0	....	8.3	8.7	13
14	12.4	5.7	6.3	3.4	....	9.1	9.5	14
15	13.3	6.2	6.7	3.9	....	9.9	10.4	15
16	14.2	6.6	7.2	4.3	....	10.6	11.2	16
17	15.1	7.0	7.6	4.8	....	11.4	12.0	17
18	16.0	7.5	8.1	5.2	....	12.2	12.9	18
19	16.9	7.9	8.5	5.7	....	13.0	13.7	19
20	17.8	8.3	8.9	6.1	....	13.8	14.6	20
21	18.7	8.7	9.4	6.6	....	14.6	15.4	21
22	19.5	9.2	9.8	7.0	....	15.4	16.2	22
23	20.4	9.6	10.3	7.5	....	16.2	17.1	23
24	21.3	10.0	10.7	7.9	....	17.0	17.9	24
25	22.2	10.5	11.2	8.4	....	17.8	18.7	25
26	23.1	10.9	11.6	8.8	....	18.6	19.6	26
27	24.0	11.3	12.0	9.3	....	19.4	20.4	27
28	24.9	11.8	12.5	9.7	....	20.2	21.2	28
29	25.8	12.2	12.9	10.2	....	21.0	22.1	29
30	26.6	12.6	13.4	10.7	4.3	21.8	22.9	30
31	27.5	13.1	13.8	11.1	4.7	22.6	23.7	31
32	28.4	13.5	14.3	11.6	5.2	23.3	24.6	32
33	29.3	13.9	14.7	12.0	5.6	24.1	25.4	33
34	30.2	14.3	15.2	12.5	6.1	24.9	26.2	34
35	31.1	14.8	15.6	12.9	6.5	25.7	27.1	35
36	32.0	15.2	16.1	13.4	7.0	26.5	27.9	36
37	32.9	15.6	16.5	13.8	7.4	27.3	28.7	37
38	33.8	16.1	16.9	14.3	7.9	28.1	29.6	38
39	34.6	16.5	17.4	14.7	8.4	28.9	30.4	39
40	35.5	16.9	17.8	15.2	8.8	29.7	31.3	40
41	36.4	17.4	18.3	15.6	9.3	30.5	32.1	41
42	37.3	17.8	18.7	16.1	9.7	31.3	32.9	42
43	38.2	18.2	19.2	16.6	10.2	32.1	33.8	43
44	39.1	18.7	19.6	17.0	10.7	32.9	34.6	44
45	40.0	19.1	20.1	17.5	11.1	33.7	35.4	45
46	40.9	19.6	20.5	17.9	11.6	34.4	36.3	46
47	41.7	20.0	21.0	18.4	12.0	35.2	37.1	47
48	42.6	20.4	21.4	18.8	12.5	36.0	37.9	48
49	43.5	20.9	21.9	19.3	12.9	36.8	38.8	49
50	44.4	21.3	22.3	19.7	13.4	37.6	39.6	50
51	45.3	21.7	22.8	20.2	13.9	38.4	40.4	51
52	46.2	22.2	23.2	20.7	14.3	39.2	41.3	52
53	47.1	22.6	23.7	21.1	14.8	40.0	42.1	53
54	48.0	23.0	24.1	21.6	15.2	40.8	42.9	54
55	48.9	23.5	24.6	22.0	15.7	41.6	43.8	55
56	49.7	23.9	25.0	22.5	16.2	42.4	44.6	56
57	50.6	24.3	25.5	22.9	16.6	43.2	45.4	57
58	51.5	24.8	25.9	23.4	17.1	44.0	46.3	58
59	52.4	25.2	26.4	23.9	17.5	44.8	47.1	59

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE,  
INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRM. AND  
2 GRM. TOTAL SUGAR), AND MALTOSE (ANHYDROUS AND  
CRYSTALLISED)

(For Lactose Figures see pages 457 to 460)—Continued  
(Expressed in milligrams)

Cuprous oxide (Cu <sub>2</sub> O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and sucrose		Maltose		Cuprous oxide (Cu <sub>2</sub> O)
				0.4 grm. total sugar	2 grm. total sugar	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> + H <sub>2</sub> O	
60	53.3	25.6	26.8	24.3	18.0	45.6	48.0	60
61	54.2	26.1	27.3	24.8	18.5	46.3	48.8	61
62	55.1	26.5	27.7	25.2	18.9	47.1	49.6	62
63	56.0	27.0	28.2	25.7	19.4	47.9	50.5	63
64	56.8	27.4	28.6	26.2	19.8	48.7	51.3	64
65	57.7	27.8	29.1	26.6	20.3	49.5	52.1	65
66	58.6	28.3	29.5	27.1	20.8	50.3	53.0	66
67	59.5	28.7	30.0	27.5	21.2	51.1	53.8	67
68	60.4	29.2	30.4	28.0	21.7	51.9	54.6	68
69	61.3	29.6	30.9	28.5	22.2	52.7	55.5	69
70	62.2	30.0	31.3	28.9	22.6	53.5	56.3	70
71	63.1	30.5	31.8	29.4	23.1	54.3	57.1	71
72	64.0	30.9	32.3	29.8	23.5	55.1	58.0	72
73	64.8	31.4	32.7	30.3	24.0	55.9	58.8	73
74	65.7	31.8	33.2	30.8	24.5	56.7	59.6	74
75	66.6	32.2	33.6	31.2	24.9	57.5	60.5	75
76	67.5	32.7	34.1	31.7	25.4	58.2	61.3	76
77	68.4	33.1	34.5	32.1	25.9	59.0	62.1	77
78	69.3	33.6	35.0	32.6	26.3	59.8	63.0	78
79	70.2	34.0	35.4	33.1	26.8	60.6	63.8	79
80	71.1	34.4	35.9	33.5	27.3	61.4	64.6	80
81	71.9	34.9	36.3	34.0	27.7	62.2	65.5	81
82	72.8	35.3	36.8	34.5	28.2	63.0	66.3	82
83	73.7	35.8	37.3	34.9	28.6	63.8	67.1	83
84	74.6	36.2	37.7	35.4	29.1	64.6	68.0	84
85	75.5	36.7	38.2	35.8	29.6	65.4	68.8	85
86	76.4	37.1	38.6	36.3	30.0	66.2	69.7	86
87	77.3	37.5	39.1	36.8	30.5	67.0	70.5	87
88	78.2	38.0	39.5	37.2	31.0	67.8	71.3	88
89	79.1	38.4	40.0	37.7	31.4	68.5	72.2	89
90	80.0	38.9	40.4	38.2	31.9	69.3	73.0	90
91	80.8	39.3	40.9	38.6	32.4	70.1	73.8	91
92	81.7	39.8	41.4	39.1	32.8	70.9	74.7	92
93	82.6	40.2	41.8	39.6	33.3	71.7	75.5	93
94	83.5	40.6	42.3	40.0	33.8	72.5	76.3	94
95	84.4	41.1	42.7	40.5	34.2	73.3	77.2	95
96	85.3	41.5	43.2	41.0	34.7	74.1	78.0	96
97	86.2	42.0	43.7	41.4	35.2	74.9	78.8	97
98	87.1	42.4	44.1	41.9	35.6	75.7	79.7	98
99	87.9	42.9	44.6	42.4	36.1	76.5	80.5	99
100	88.8	43.3	45.0	42.8	36.6	77.3	81.3	100
101	89.7	43.8	45.5	43.3	37.0	78.1	82.2	101
102	90.6	44.2	46.0	43.8	37.5	78.8	83.0	102
103	91.5	44.7	46.4	44.2	38.0	79.6	83.8	103
104	92.4	45.1	46.9	44.7	38.5	80.4	84.7	104
105	93.3	45.5	47.3	45.2	38.9	81.2	85.5	105
106	94.2	46.0	47.8	45.6	39.4	82.0	86.3	106
107	95.0	46.4	48.3	46.1	39.9	82.8	87.2	107
108	95.9	46.9	48.7	46.4	40.3	83.6	88.0	108
109	96.8	47.3	49.2	47.0	40.8	84.4	88.8	109

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRM. AND 2 GRM. TOTAL SUGAR), AND MALTOSE (ANHYDROUS AND CRYSTALLISED)

(For Lactose Figures see pages 457 to 460)—Continued  
(Expressed in milligrams)

Cuprous oxide (Cu <sub>2</sub> O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and sucrose		Maltose		Cuprous oxide (Cu <sub>2</sub> O)
				0.4 grm. total sugar	2 grm. total sugar	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> + H <sub>2</sub> O	
110	97.7	47.8	49.6	47.5	41.3	85.2	89.7	110
111	98.6	48.2	50.1	48.0	41.7	86.0	90.5	111
112	99.5	48.7	50.6	48.4	42.2	86.8	91.3	112
113	100.4	49.1	51.0	48.9	42.7	87.6	92.2	113
114	101.3	49.6	51.5	49.4	43.2	88.4	93.0	114
115	102.2	50.0	51.9	49.8	43.6	89.2	93.9	115
116	103.0	50.5	52.4	50.3	44.1	90.0	94.7	116
117	103.9	50.9	52.9	50.8	44.6	90.7	95.5	117
118	104.8	51.4	53.3	51.2	45.0	91.5	96.4	118
119	105.7	51.8	53.8	51.7	45.5	92.3	97.2	119
120	106.6	52.3	54.3	52.2	46.0	93.1	98.0	120
121	107.5	52.7	54.7	52.7	46.5	93.9	98.9	121
122	108.4	53.2	55.2	53.1	46.9	94.7	99.7	122
123	109.3	53.6	55.7	53.6	47.4	95.5	100.5	123
124	110.1	54.1	56.1	54.1	47.9	96.3	101.4	124
125	111.0	54.5	56.6	54.5	48.3	97.1	102.2	125
126	111.9	55.0	57.0	55.0	48.8	97.9	103.0	126
127	112.8	55.4	57.5	55.5	49.3	98.7	103.9	127
128	113.7	55.9	58.0	55.9	49.8	99.4	104.7	128
129	114.6	56.3	58.4	56.4	50.2	100.2	105.5	129
130	115.5	56.8	58.9	56.9	50.7	101.0	106.4	130
131	116.4	57.2	59.4	57.4	51.2	101.8	107.2	131
132	117.3	57.7	59.8	57.8	51.7	102.6	108.0	132
133	118.1	58.1	60.3	58.3	52.1	103.4	108.9	133
134	119.0	58.6	60.8	58.8	52.6	104.2	109.7	134
135	119.9	59.0	61.2	59.3	53.1	105.0	110.5	135
136	120.8	59.5	61.7	59.7	53.6	105.8	111.4	136
137	121.7	60.0	62.2	60.2	54.0	106.6	112.2	137
138	122.6	60.4	62.6	60.7	54.5	107.4	113.0	138
139	123.5	60.9	63.1	61.2	55.0	108.2	113.9	139
140	124.4	61.3	63.6	61.6	55.5	109.0	114.7	140
141	125.2	61.8	64.0	62.1	55.9	109.8	115.5	141
142	126.1	62.2	64.5	62.6	56.4	110.5	116.4	142
143	127.0	62.7	65.0	63.1	56.9	111.3	117.2	143
144	127.9	63.1	65.4	63.5	57.4	112.1	118.0	144
145	128.8	63.6	65.9	64.0	57.8	112.9	118.9	145
146	129.7	64.0	66.4	64.5	58.3	113.7	119.7	146
147	130.6	64.5	66.9	65.0	58.8	114.5	120.5	147
148	131.5	65.0	67.3	65.4	59.3	115.3	121.4	148
149	132.4	65.4	67.8	65.9	59.7	116.1	122.2	149
150	133.2	65.9	68.3	66.4	60.2	116.9	123.0	150
151	134.1	66.3	68.7	66.9	60.7	117.7	123.9	151
152	135.0	66.8	69.2	67.3	61.2	118.5	124.7	152
153	135.9	67.2	69.7	67.8	61.7	119.3	125.5	153
154	136.8	67.7	70.1	68.3	62.1	120.0	126.4	154
155	137.7	68.2	70.6	68.8	62.6	120.8	127.2	155
156	138.6	68.6	71.1	69.2	63.1	121.6	128.0	156
157	139.5	69.1	71.6	69.7	63.6	122.4	128.9	157
158	140.3	69.5	72.0	70.2	64.1	123.2	129.7	158
159	141.2	70.0	72.5	70.7	64.5	124.0	130.5	159

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE,  
INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRM. AND  
2 GRM. TOTAL SUGAR), AND MALTOSE (ANHYDROUS AND  
CRYSTALLISED)

(For Lactose Figures see pages 457 to 460)—Continued  
(Expressed in milligrams)

Cuprous oxide (Cu <sub>2</sub> O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and sucrose		Maltose		Cuprous oxide (Cu <sub>2</sub> O)
				0.4 grm. total sugar	2 grm. total sugar	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> +H <sub>2</sub> O	
160	142.1	70.4	73.0	71.2	65.0	124.8	131.4	160
161	143.0	70.9	73.4	71.6	65.5	125.6	132.2	161
162	143.9	71.4	73.9	72.1	66.0	126.4	133.0	162
163	144.8	71.8	74.4	72.6	66.5	127.2	133.9	163
164	145.7	72.3	74.9	73.1	66.9	128.0	134.7	164
165	146.6	72.8	75.3	73.6	67.4	128.8	135.5	165
166	147.5	73.2	75.8	74.0	67.9	129.6	136.4	166
167	148.3	73.7	76.3	74.5	68.4	130.3	137.2	167
168	149.2	74.1	76.8	75.0	68.9	131.1	138.0	168
169	150.1	74.6	77.2	75.5	69.3	131.9	138.9	169
170	151.0	75.1	77.7	76.0	69.8	132.7	139.7	170
171	151.9	75.5	78.2	76.4	70.3	133.5	140.5	171
172	152.8	76.0	78.7	76.9	70.8	134.3	141.4	172
173	153.7	76.4	79.1	77.4	71.3	135.1	142.2	173
174	154.6	76.9	79.6	77.9	71.7	135.9	143.0	174
175	155.5	77.4	80.1	78.4	72.2	136.7	143.9	175
176	156.3	77.8	80.6	78.8	72.7	137.5	144.7	176
177	157.2	78.3	81.0	79.3	73.2	138.3	145.5	177
178	158.1	78.8	81.5	79.8	73.7	139.1	146.4	178
179	159.0	79.2	82.0	80.3	74.2	139.8	147.2	179
180	159.9	79.7	82.5	80.8	74.6	140.6	148.0	180
181	160.8	80.1	82.9	81.3	75.1	141.4	148.9	181
182	161.7	80.6	83.4	81.7	75.6	142.2	149.7	182
183	162.6	81.1	83.9	82.2	76.1	143.0	150.5	183
184	163.4	81.5	84.4	82.7	76.6	143.8	151.4	184
185	164.3	82.0	84.9	83.2	77.1	144.6	152.2	185
186	165.2	82.5	85.3	83.7	77.6	145.4	153.0	186
187	166.1	82.9	85.8	84.2	78.0	146.2	153.9	187
188	167.0	83.4	86.3	84.6	78.5	147.0	154.7	188
189	167.9	83.9	86.8	85.1	79.0	147.8	155.5	189
190	168.8	84.3	87.2	85.6	79.5	148.6	156.4	190
191	169.7	84.8	87.7	86.1	80.0	149.3	157.2	191
192	170.5	85.3	88.2	86.6	80.5	150.1	158.0	192
193	171.4	85.7	88.7	87.1	81.0	150.9	158.9	193
194	172.3	86.2	89.2	87.6	81.4	151.7	159.7	194
195	173.2	86.7	89.6	88.0	81.9	152.5	160.5	195
196	174.1	87.1	90.1	88.5	82.4	153.3	161.4	196
197	175.0	87.6	90.6	89.0	82.9	154.1	162.2	197
198	175.9	88.1	91.1	89.5	83.4	154.9	163.0	198
199	176.8	88.5	91.6	90.0	83.9	155.7	163.9	199
200	177.7	89.0	92.0	90.5	84.4	156.5	164.7	200
201	178.5	89.5	92.5	91.0	84.8	157.3	165.5	201
202	179.4	89.9	93.0	91.4	85.3	158.1	166.4	202
203	180.3	90.4	93.5	91.9	85.8	158.8	167.2	203
204	181.2	90.9	94.0	92.4	86.3	159.6	168.0	204
205	182.1	91.4	94.5	92.9	86.8	160.4	168.9	205
206	183.0	91.8	94.9	93.4	87.3	161.2	169.7	206
207	183.9	92.3	95.4	93.9	87.8	162.0	170.5	207
208	184.8	92.8	95.9	94.4	88.3	162.8	171.4	208
209	185.6	93.2	96.4	94.9	88.8	163.6	172.2	209

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE,  
INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRM. AND  
2 GRM. TOTAL SUGAR), AND MALTOSE (ANHYDROUS AND  
CRYSTALLISED)

(For Lactose Figures see pages 457 to 460)—Continued  
(Expressed in milligrams)

Cuprous oxide (Cu <sub>2</sub> O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and sucrose		Maltose		Cuprous oxide (Cu <sub>2</sub> O)
				0.4 grm. total sugar	2 grm. total sugar	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> + H <sub>2</sub> O	
210	186.5	93.7	96.9	95.4	89.2	164.4	173.0	210
211	187.4	94.2	97.4	95.8	89.7	165.2	173.8	211
212	188.3	94.6	97.8	96.3	90.2	166.0	174.7	212
213	189.2	95.1	98.3	96.8	90.7	166.8	175.5	213
214	190.1	95.6	98.8	97.3	91.2	167.5	176.4	214
215	191.0	96.1	99.3	97.8	91.7	168.3	177.2	215
216	191.9	96.5	99.8	98.3	92.2	169.1	178.0	216
217	192.8	97.0	100.3	98.8	92.7	169.9	178.9	217
218	193.6	97.5	100.8	99.3	93.2	170.7	179.7	218
219	194.5	98.0	101.2	99.8	93.7	171.5	180.5	219
220	195.4	98.4	101.7	100.3	94.2	172.3	181.4	220
221	196.3	98.9	102.2	100.8	94.7	173.1	182.2	221
222	197.2	99.4	102.7	101.2	95.1	173.9	183.0	222
223	198.1	99.9	103.2	101.7	95.6	174.7	183.9	223
224	199.0	100.3	103.7	102.2	96.1	175.5	184.7	224
225	199.9	100.8	104.2	102.7	96.6	176.2	185.5	225
226	200.7	101.3	104.6	103.2	97.1	177.0	186.4	226
227	201.6	101.8	105.1	103.7	97.6	177.8	187.2	227
228	202.5	102.2	105.6	104.2	98.1	178.6	188.0	228
229	203.4	102.7	106.1	104.7	98.6	179.4	188.8	229
230	204.3	103.2	106.6	105.2	99.1	180.2	189.7	230
231	205.2	103.7	107.1	105.7	99.6	181.0	190.5	231
232	206.1	104.1	107.6	106.2	100.1	181.8	191.3	232
233	207.0	104.6	108.1	106.7	100.6	182.6	192.2	233
234	207.9	105.1	108.6	107.2	101.1	183.4	193.0	234
235	208.7	105.6	109.1	107.7	101.6	184.2	193.8	235
236	209.6	106.0	109.5	108.2	102.1	184.9	194.7	236
237	210.5	106.5	110.0	108.7	102.6	185.7	195.5	237
238	211.4	107.0	110.5	109.2	103.1	186.5	196.3	238
239	212.3	107.5	111.0	109.6	103.5	187.3	197.2	239
240	213.2	108.0	111.5	110.1	104.0	188.1	198.0	240
241	214.1	108.4	112.0	110.6	104.5	188.9	198.8	241
242	215.0	108.9	112.5	111.1	105.0	189.7	199.7	242
243	215.8	109.4	113.0	111.6	105.5	190.5	200.5	243
244	216.7	109.9	113.5	112.1	106.0	191.3	201.3	244
245	217.6	110.4	114.0	112.6	106.5	192.1	202.2	245
246	218.5	110.8	114.5	113.1	107.0	192.9	203.0	246
247	219.4	111.3	115.0	113.6	107.5	193.6	203.8	247
248	220.3	111.8	115.4	114.1	108.0	194.4	204.7	248
249	221.2	112.3	115.9	114.6	108.5	195.2	205.5	249
250	222.1	112.8	116.4	115.1	109.0	196.0	206.3	250
251	223.0	113.2	116.9	115.6	109.5	196.8	207.2	251
252	223.8	113.7	117.4	116.1	110.0	197.6	208.0	252
253	224.7	114.2	117.9	116.6	110.5	198.4	208.8	253
254	225.6	114.7	118.4	117.1	111.0	199.2	209.7	254
255	226.5	115.2	118.9	117.6	111.5	200.0	210.5	255
256	227.4	115.7	119.4	118.1	112.0	200.8	211.3	256
257	228.3	116.1	119.9	118.6	112.5	201.6	212.2	257
258	229.2	116.6	120.4	119.1	113.0	202.3	213.0	258
259	230.1	117.1	120.9	119.6	113.5	203.1	213.8	259

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE,  
INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRM. AND  
2 GRM. TOTAL SUGAR), AND MALTOSE (ANHYDROUS AND  
CRYSTALLISED)

(For Lactose Figures see pages 457 to 460)—Continued  
(Expressed in milligrams)

Cuprous oxide (Cu <sub>2</sub> O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and sucrose		Maltose		Cuprous oxide (Cu <sub>2</sub> O)
				0.4 grm. total sugar	2 grm. total sugar	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> + H <sub>2</sub> O	
260	231 0	117 6	121 4	120 1	114 0	203 9	214 7	260
261	231 8	118 1	121 9	120 6	114 5	204 7	215 5	261
262	232 7	118 6	122 4	121 1	115 0	205 5	216 3	262
263	233 6	119 0	122 9	121 6	115 5	206 3	217 2	263
264	234 5	119 5	123 4	122 1	116 0	207 1	218 0	264
265	235 4	120 0	123 9	122 6	116 5	207 9	218 8	265
266	236 3	120 5	124 4	123 1	117 0	208 7	219 7	266
267	237 2	121 0	124 9	123 6	117 5	209 5	220 5	267
268	238 1	121 5	125 4	124 1	118 0	210 3	221 3	268
269	238 9	122 0	125 9	124 6	118 5	211 0	222 1	269
270	239 8	122 5	126 4	125 1	119 0	211 8	223 0	270
271	240 7	122 9	126 9	125 6	119 5	212 6	223 8	271
272	241 6	123 4	127 4	126 2	120 0	213 4	224 6	272
273	242 5	123 9	127 9	126 7	120 6	214 2	225 5	273
274	243 4	124 4	128 4	127 2	121 1	215 0	226 3	274
275	244 3	124 9	128 9	127 7	121 6	215 8	227 1	275
276	245 2	125 4	129 4	128 2	122 1	216 6	228 0	276
277	246 1	125 9	129 9	128 7	122 6	217 4	228 8	277
278	246 9	126 4	130 4	129 2	123 1	218 2	229 6	278
279	247 8	126 9	130 9	129 7	123 6	218 9	230 5	279
280	248 7	127 3	131 4	130 2	124 1	219 7	231 3	280
281	249 6	127 8	131 9	130 7	124 6	220 5	232 1	281
282	250 5	128 3	132 4	131 2	125 1	221 3	233 0	282
283	251 4	128 8	132 9	131 7	125 6	222 1	233 8	283
284	252 3	129 3	133 4	132 2	126 1	222 9	234 6	284
285	253 2	129 8	133 9	132 7	126 6	223 7	235 5	285
286	254 0	130 3	134 4	133 2	127 1	224 5	236 3	286
287	254 9	130 8	134 9	133 7	127 6	225 3	237 1	287
288	255 8	131 3	135 4	134 3	128 1	226 1	238 0	288
289	256 7	131 8	135 9	134 8	128 6	226 9	238 8	289
290	257 6	132 3	136 4	135 3	129 2	227 6	239 6	290
291	258 5	132 7	136 9	135 8	129 7	228 4	240 5	291
292	259 4	133 2	137 4	136 3	130 2	229 2	241 3	292
293	260 3	133 7	137 9	136 8	130 7	230 0	242 1	293
294	261 2	134 2	138 4	137 3	131 2	230 8	242 9	294
295	262 0	134 7	138 9	137 8	131 7	231 6	243 8	295
296	262 9	135 2	139 4	138 3	132 2	232 4	244 6	296
297	263 8	135 7	140 0	138 8	132 7	233 2	245 4	297
298	264 7	136 2	140 5	139 4	133 2	234 0	246 3	298
299	265 6	136 7	141 0	139 9	133 7	234 8	247 1	299
300	266 5	137 2	141 5	140 4	134 2	235 5	247 9	300
301	267 4	137 7	142 0	140 9	134 8	236 3	248 8	301
302	268 3	138 2	142 5	141 4	135 3	237 1	249 6	302
303	269 1	138 7	143 0	141 9	135 8	237 9	250 4	303
304	270 0	139 2	143 5	142 4	136 3	238 7	251 3	304
305	270 9	139 7	144 0	142 9	136 8	239 5	252 1	305
306	271 8	140 2	144 5	143 4	137 3	240 3	252 9	306
307	272 7	140 7	145 0	144 0	137 8	241 1	253 8	307
308	273 6	141 2	145 5	144 5	138 3	241 9	254 6	308
309	274 5	141 7	146 0	145 0	138 8	242 7	255 4	309

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE,  
INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRM. AND  
2 GRM. TOTAL SUGAR), AND MALTOSE (ANHYDROUS AND  
CRYSTALLISED)

(For Lactose Figures see pages 457 to 460)—Continued  
(Expressed in milligrams)

Cuprous oxide (Cu <sub>2</sub> O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and sucrose		Maltose		Cuprous oxide (Cu <sub>2</sub> O)
				0.4 grm. total sugar	2 grm. total sugar	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> + H <sub>2</sub> O	
310	275.4	142.2	146.6	145.5	139.4	243.5	256.3	310
311	276.3	142.7	147.1	146.0	139.9	244.2	257.1	311
312	277.1	143.2	147.6	146.5	140.4	245.0	257.9	312
313	278.0	143.7	148.1	147.0	140.9	245.8	258.8	313
314	278.9	144.2	148.6	147.6	141.4	246.6	259.6	314
315	279.8	144.7	149.1	148.1	141.9	247.4	260.4	315
316	280.7	145.2	149.6	148.6	142.4	248.2	261.2	316
317	281.6	145.7	150.1	149.1	143.0	249.0	262.1	317
318	282.5	146.2	150.7	149.6	143.5	249.8	262.9	318
319	283.4	146.7	151.2	150.1	144.0	250.6	263.7	319
320	284.2	147.2	151.7	150.7	144.5	251.3	264.6	320
321	285.1	147.7	152.2	151.2	145.0	252.1	265.4	321
322	286.0	148.2	152.7	151.7	145.5	252.9	266.2	322
323	286.9	148.7	153.2	152.2	146.0	253.7	267.1	323
324	287.8	149.2	153.7	152.7	146.6	254.5	267.9	324
325	288.7	149.7	154.3	153.2	147.1	255.3	268.7	325
326	289.6	150.2	154.8	153.8	147.6	256.1	269.6	326
327	290.5	150.7	155.3	154.3	148.1	256.9	270.4	327
328	291.4	151.2	155.8	154.8	148.6	257.7	271.2	328
329	292.2	151.7	156.3	155.3	149.1	258.5	272.1	329
330	293.1	152.2	156.8	155.8	149.7	259.3	272.9	330
331	294.0	152.7	157.3	156.4	150.2	260.0	273.7	331
332	294.9	153.2	157.9	156.9	150.7	260.8	274.6	332
333	295.8	153.7	158.4	157.4	151.2	261.6	275.4	333
334	296.7	154.2	158.9	157.9	151.7	262.4	276.2	334
335	297.6	154.7	159.4	158.4	152.3	263.2	277.0	335
336	298.5	155.2	159.9	159.0	152.8	264.0	277.9	336
337	299.3	155.8	160.5	159.5	153.3	264.8	278.7	337
338	300.2	156.3	161.0	160.0	153.8	265.6	279.5	338
339	301.1	156.8	161.5	160.5	154.3	266.4	280.4	339
340	302.0	157.3	162.0	161.0	154.8	267.1	281.2	340
341	302.9	157.8	162.5	161.6	155.4	267.9	282.0	341
342	303.8	158.3	163.1	162.1	155.9	268.7	282.9	342
343	304.7	158.8	163.6	162.6	156.4	269.5	283.7	343
344	305.6	159.3	164.1	163.1	156.9	270.3	284.5	344
345	306.5	159.8	164.6	163.7	157.5	271.1	285.4	345
346	307.3	160.3	165.1	164.2	158.0	271.9	286.2	346
347	308.2	160.8	165.7	164.7	158.5	272.7	287.0	347
348	309.1	161.4	166.2	165.2	159.0	273.5	287.9	348
349	310.0	161.9	166.7	165.7	159.5	274.3	288.7	349
350	310.9	162.4	167.2	166.3	160.1	275.0	289.5	350
351	311.8	162.9	167.7	166.8	160.6	275.8	290.4	351
352	312.7	163.4	168.3	167.3	161.1	276.6	291.2	352
353	313.6	163.9	168.8	167.8	161.6	277.4	292.0	353
354	314.4	164.4	169.3	168.4	162.2	278.2	292.8	354
355	315.3	164.9	169.8	168.9	162.7	279.0	293.7	355
356	316.2	165.4	170.4	169.4	163.2	279.8	294.5	356
357	317.1	166.0	170.9	170.0	163.7	280.6	295.3	357
358	318.0	166.5	171.4	170.5	164.3	281.4	296.2	358
359	318.9	167.0	171.9	171.0	164.8	282.2	297.0	359

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE,  
INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRM AND  
2 GRM. TOTAL SUGAR), AND MALTOSE (ANHYDROUS AND  
CRYSTALLISED)

(For Lactose Figures see pages 457 to 460)—Continued  
(Expressed in milligrams)

Cuprous oxide (Cu <sub>2</sub> O)	Copper (Cu)	Dextrose (d glucose)	Invert sugar	Invert sugar and sucrose		Maltose		Cuprous oxide (Cu <sub>2</sub> O)
				0.4 grm. total sugar	2 grm total sugar	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> + H <sub>2</sub> O	
360	319.8	167.5	172.5	171.5	165.3	282.9	297.8	360
361	320.7	168.0	173.0	172.1	165.8	283.7	298.7	361
362	321.6	168.5	173.5	172.6	166.4	284.5	299.5	362
363	322.4	169.0	174.0	173.1	166.9	285.3	300.3	363
364	323.3	169.6	174.6	173.7	167.4	286.1	301.2	364
365	324.2	170.1	175.1	174.2	167.9	286.9	302.0	365
366	325.1	170.6	175.6	174.7	168.5	287.7	302.8	366
367	326.0	171.1	176.1	175.2	169.0	288.5	303.6	367
368	326.9	171.6	176.7	175.8	169.5	289.3	304.5	368
369	327.8	172.1	177.2	176.3	170.0	290.0	305.3	369
370	328.7	172.7	177.7	176.8	170.6	290.8	306.1	370
371	329.5	173.2	178.3	177.4	171.1	291.6	307.0	371
372	330.4	173.7	178.8	177.9	171.6	292.4	307.8	372
373	331.3	174.2	179.3	178.4	172.2	293.2	308.6	373
374	332.2	174.7	179.8	179.0	172.7	294.0	309.5	374
375	333.1	175.3	180.4	179.5	173.2	294.8	310.3	375
376	334.0	175.8	180.9	180.0	173.7	295.6	311.1	376
377	334.9	176.3	181.4	180.6	174.3	296.4	312.0	377
378	335.8	176.8	182.0	181.1	174.8	297.2	312.8	378
379	336.7	177.3	182.5	181.6	175.3	297.9	313.6	379
380	337.5	177.9	183.0	182.1	175.9	298.7	314.5	380
381	338.4	178.4	183.6	182.7	176.4	299.5	315.3	381
382	339.3	178.9	184.1	183.2	176.9	300.3	316.1	382
383	340.2	179.4	184.6	183.8	177.5	301.1	316.9	383
384	341.1	180.0	185.2	184.3	178.0	331.9	317.8	384
385	342.0	180.5	185.7	184.8	178.5	302.7	318.6	385
386	342.9	181.0	186.2	185.4	179.1	303.5	319.4	386
387	343.8	181.5	186.8	185.9	179.6	304.2	320.3	387
388	344.6	182.0	187.3	186.4	180.1	305.0	321.1	388
389	345.5	182.6	187.8	187.0	180.6	305.8	321.9	389
390	346.4	183.1	188.4	187.5	181.2	306.6	322.8	390
391	347.3	183.6	188.9	188.0	181.7	307.4	323.6	391
392	348.2	184.1	189.4	188.6	182.3	308.2	324.4	392
393	349.1	184.7	190.0	189.1	182.8	309.0	325.2	393
394	350.0	185.2	190.5	189.7	183.3	309.8	326.1	394
395	350.9	185.7	191.0	190.2	183.9	310.6	326.9	395
396	351.8	186.2	191.6	190.7	184.4	311.4	327.7	396
397	352.6	186.8	192.1	191.3	184.9	312.1	328.6	397
398	353.5	187.3	192.7	191.8	185.5	312.9	329.4	398
399	354.4	187.8	193.2	192.3	186.0	313.7	330.2	399
400	355.3	188.4	193.7	192.9	186.5	314.5	331.1	400
401	356.2	188.9	194.3	193.4	187.1	315.3	331.9	401
402	357.1	189.4	194.8	194.0	187.6	316.1	332.7	402
403	358.0	189.9	195.4	194.5	188.1	316.9	333.6	403
404	358.9	190.5	195.9	195.0	188.7	317.7	334.4	404
405	359.7	191.0	196.4	195.6	189.2	318.5	335.2	405
406	360.6	191.5	197.0	196.1	189.8	319.2	336.0	406
407	361.5	192.1	197.5	196.7	190.3	320.0	336.9	407
408	362.4	192.6	198.1	197.2	190.8	320.8	337.7	408
409	363.3	193.1	198.6	197.7	191.4	321.6	338.5	409

TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE,  
INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRM. AND  
2 GRM. TOTAL SUGAR), AND MALTOSE (ANHYDROUS AND  
CRYSTALLISED)

(For Lactose Figures see pages 457 to 460)—Continued  
(Expressed in milligrams)

Cuprous oxide (Cu <sub>2</sub> O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and sucrose		Maltose		Cuprous oxide (Cu <sub>2</sub> O)
				0.4 grm. total sugar	2 grm. total sugar	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> + H <sub>2</sub> O	
410	364.2	193.7	199.1	198.3	191.9	322.4	339.4	410
411	365.1	194.2	199.7	198.8	192.5	323.2	340.2	411
412	366.0	194.7	200.2	199.4	193.0	324.0	341.0	412
413	366.9	195.2	200.8	199.9	193.5	324.8	341.9	413
414	367.7	195.8	201.3	200.5	194.1	325.6	342.7	414
415	368.6	196.3	201.8	201.0	194.6	326.3	343.5	415
416	369.5	196.8	202.4	201.6	195.2	327.1	344.4	416
417	370.4	197.4	202.9	202.1	195.7	327.9	345.2	417
418	371.3	197.9	203.5	202.6	196.2	328.7	346.0	418
419	372.2	198.4	204.0	203.2	196.8	329.5	346.8	419
420	373.1	199.0	204.6	203.7	197.3	330.3	347.7	420
421	374.0	199.5	205.1	204.3	197.9	331.1	348.5	421
422	374.8	200.1	205.7	204.8	198.4	331.9	349.3	422
423	375.7	200.6	206.2	205.4	198.9	332.7	350.2	423
424	376.6	201.1	206.7	205.9	199.5	333.4	351.0	424
425	377.5	201.7	207.3	206.5	200.0	334.2	351.8	425
426	378.4	202.2	207.8	207.0	200.6	335.0	352.7	426
427	379.3	202.8	208.4	207.6	201.1	335.8	353.5	427
428	380.2	203.3	208.9	208.1	201.7	336.6	354.3	428
429	381.1	203.8	209.5	208.7	202.2	337.4	355.1	429
430	382.0	204.4	210.0	209.2	202.7	338.2	356.0	430
431	382.8	204.9	210.6	209.8	203.3	339.0	356.8	431
432	383.7	205.5	211.1	210.3	203.8	339.7	357.6	432
433	384.6	206.0	211.7	210.9	204.4	340.5	358.5	433
434	385.5	206.5	212.2	211.4	204.9	341.3	359.3	434
435	386.4	207.1	212.8	212.0	205.5	342.1	360.1	435
436	387.3	207.6	213.3	212.5	206.0	342.9	361.0	436
437	388.2	208.2	213.9	213.1	206.6	343.7	361.8	437
438	389.1	208.7	214.4	213.6	207.1	344.5	362.6	438
439	390.0	209.2	215.0	214.2	207.7	345.3	363.4	439
440	390.8	209.8	215.5	214.7	208.2	346.1	364.3	440
441	391.7	210.3	216.1	215.3	208.8	346.8	365.1	441
442	392.6	210.9	216.6	215.8	209.3	347.6	365.9	442
443	393.5	211.4	217.2	216.4	209.9	348.4	366.8	443
444	394.4	212.0	217.8	216.9	210.4	349.2	367.6	444
445	395.3	212.5	218.3	217.5	211.0	350.0	368.4	445
446	396.2	213.1	218.9	218.0	211.5	350.8	369.3	446
447	397.1	213.6	219.4	218.6	212.1	351.6	370.1	447
448	397.9	214.1	220.0	219.1	212.6	352.4	370.9	448
449	398.8	214.7	220.5	219.7	213.2	353.2	371.7	449
450	399.7	215.2	221.1	220.2	213.7	353.9	372.6	450
451	400.6	215.8	221.6	220.8	214.3	354.7	373.4	451
452	401.5	216.3	222.2	221.4	214.8	355.5	374.2	452
453	402.4	216.9	222.8	221.9	215.4	356.3	375.1	453
454	403.3	217.4	223.3	222.5	215.9	357.1	375.9	454
455	404.2	218.0	223.9	223.0	216.5	357.9	376.7	455
456	405.1	218.5	224.4	223.6	217.0	358.7	377.6	456
457	405.9	219.1	225.0	224.1	217.6	359.5	378.4	457
458	406.8	219.6	225.5	224.7	218.1	360.3	379.2	458
459	407.7	220.2	226.1	225.3	218.7	361.0	380.0	459

TABLE FOR CALCULATING DEXTROSE INVERT SUGAR ALONE,  
INVERT SUGAR IN THE PRESENCE OF SUCROSE (0.4 GRM. AND  
2 GRM. TOTAL SUGAR), AND MALTOSE (ANHYDROUS AND  
CRYSTALLISED)

(For Lactose Figures see pages 457 to 460)—*Continued*  
(Expressed in milligrams)

Cuprous oxide (Cu <sub>2</sub> O)	Copper (Cu)	Dextrose (d-glucose)	Invert sugar	Invert sugar and sucrose		Maltose		Cuprous oxide (Cu <sub>2</sub> C)
				0.4 grm. total sugar	2 grm. total sugar	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> + H <sub>2</sub> O	
460	408.6	220.7	226.7	225.8	219.2	361.8	380.9	460
461	409.5	221.3	227.2	226.4	219.8	362.6	381.7	461
462	410.4	221.8	227.8	226.9	220.3	363.4	382.5	462
463	411.3	222.4	228.3	227.5	220.9	364.2	383.4	463
464	412.2	222.9	228.9	228.1	221.4	365.0	384.2	464
465	413.0	223.5	229.5	228.6	222.0	365.8	385.0	465
466	413.9	224.0	230.0	229.2	222.5	366.6	385.9	466
467	414.8	224.6	230.6	229.7	223.1	367.3	386.7	467
468	415.7	225.1	231.2	230.3	223.7	368.1	387.5	468
469	416.6	225.7	231.7	230.9	224.2	368.9	388.3	469
470	417.5	226.2	232.3	231.4	224.8	369.7	389.2	470
471	418.4	226.8	232.8	232.0	225.3	370.5	390.0	471
472	419.3	227.4	233.4	232.5	225.9	371.3	390.8	472
473	420.2	227.9	234.0	233.1	226.4	372.1	391.7	473
474	421.0	228.5	234.5	233.7	227.0	372.9	392.5	474
475	421.9	229.0	235.1	234.2	227.6	373.7	393.3	475
476	422.8	229.6	235.7	234.8	228.1	374.4	394.2	476
477	423.7	230.1	236.2	235.4	228.7	375.2	395.0	477
478	424.6	230.7	236.8	235.9	229.2	376.0	395.8	478
479	425.5	231.3	237.4	236.5	229.8	376.8	396.6	479
480	426.4	231.8	237.9	237.1	230.3	377.6	397.5	480
481	427.3	232.4	238.5	237.6	230.9	378.4	398.3	481
482	428.1	232.9	239.1	238.2	231.5	379.2	399.1	482
483	429.0	233.5	239.6	238.8	232.0	380.0	400.0	483
484	429.9	234.1	240.2	239.3	232.6	380.7	400.8	484
485	430.8	234.6	240.8	239.9	233.2	381.5	401.6	485
486	431.7	235.2	241.4	240.5	233.7	382.3	402.4	486
487	432.6	235.7	241.9	241.0	234.3	383.1	403.3	487
488	433.5	236.3	242.5	241.6	234.8	383.9	404.1	488
489	434.4	236.9	243.1	242.2	235.4	384.7	404.9	489
490	435.3	237.4	243.6	242.7	236.0	385.5	405.8	490

is absent. In the tables following, the absence of sucrose is assumed except in the two columns under invert sugar, where one for mixtures of invert sugar and sucrose (0.4 grm. of total sugar in 50 c.c. of solution) and one for invert sugar and sucrose when the 50 c.c. of solution contains 2 grm. of total sugar are given, in addition to the column for invert sugar alone.

**Remarks.**—It will be noticed that in this method it is recommended that the precipitate obtained should be weighed as cuprous oxide. This is undesirable for the reasons given on page 395, unless

comparatively pure sugar solutions are being worked with. With the solutions obtained from ordinary plant products and from conversions by means of enzymes, etc., considerable error is undoubtedly incurred unless the copper is weighed as cupric oxide.

### **Estimation of Small Quantities of Reducing Sugars in Presence of a Large Quantity of Sucrose**

It is well known that when large quantities of sucrose are present, the values obtained for the reducing sugars, present in relatively small proportion, are considerably increased; this is probably due to the fact that, under the ordinary conditions of working, when the action takes place at the boiling point, traces of the sucrose are hydrolysed and so increase the actual amount of invert sugar present.

It will be seen from Munson and Walker's table that their method makes no provision for cases where, in mixtures of sucrose and invert sugar, the proportion of the latter is less than 0.21 % (*i. e.*, 4.3 mg. of invert sugar in 2 grm. of the mixture). Since raw beet sugars generally, and high-grade raw cane sugars sometimes, contain less than this proportion of invert sugar, one of the following methods may be used in such cases.

**1. Herzfeld's Method for Materials Containing 1% or Less of Invert Sugar and 99 % or More of Sucrose.**—25 grm. of the sample are dissolved in water, clarified with basic lead acetate, made up to 100 c.c. and filtered; 60 c.c. of the filtrate are treated with sodium carbonate or sulphate to remove lead, made up to 75 c.c. and filtered. 50 c.c. of the filtrate (= 10 grm. of the sample) are mixed with 50 c.c. of Fehling's solution (Soxhlet's modification, see p. 392) in a boiling flask (or covered beaker of 250 c.c. capacity), and heated at such a rate that approximately 4 minutes are required to bring the mixture to boiling, the boiling then being continued for exactly 2 minutes. The flask is at once removed from the flame, 100 c.c. of cold, recently boiled distilled water are added (in order to prevent any further precipitation of cuprous oxide) and the whole is filtered through a weighed, asbestos-packed Soxhlet tube.<sup>1</sup> The precipitate is thoroughly washed with hot water, then with alcohol and ether, and dried in the oven. The tube is then heated over a burner,

<sup>1</sup> Platinum, silica or porcelain Gooch crucible.

to convert the cuprous to cupric oxide and oxidise any organic matter, and the cupric oxide is finally reduced to metallic copper by heating in a current of hydrogen. The percentage of invert sugar in the sample is found from the following table:

HERZFELD'S TABLE FOR THE ESTIMATION OF INVERT SUGAR IN MATERIALS CONTAINING 1% OR LESS OF INVERT SUGAR AND 99% OR MORE OF SUCROSE

Copper reduced by 10 grm. of material, milligrams	Invert sugar, %	Copper reduced by 10 grm. of material, milligrams	Invert sugar, %	Copper reduced by 10 grm. of material, milligrams	Invert sugar, %
50	0.05	120	0.40	190	0.79
55	0.07	125	0.43	195	0.82
60	0.09	130	0.45	200	0.85
65	0.11	135	0.48	205	0.88
70	0.14	140	0.51	210	0.90
75	0.16	145	0.53	215	0.93
80	0.19	150	0.56	220	0.96
85	0.21	155	0.59	225	0.99
90	0.24	160	0.62	230	1.02
95	0.27	165	0.65	235	1.05
100	0.30	170	0.68	240	1.07
105	0.32	175	0.71	245	1.10
110	0.35	180	0.74		
115	0.38	185	0.76		

Pellet (*Bull. Assoc. Chim. Sucr.*, 1913, 31, 182) has made a special study of this question and points out that the amount of reduction obtained on heating Fehling's solution with solutions of sucrose varies with: (1) the time of boiling; (2) the proportion of Fehling's solution relative to the sugar; (3) the quantity of sucrose; (4) the proportion of alkaline hydroxide in the Fehling's solution.

The effect of sucrose on Fehling's solution according to Pellet is obviated by carrying out the heating, not at 100° as is usual, but at 60°-62°; at this temperature there is not a trace of cuprous oxide formed when 10 grm. of pure sucrose are heated for 10 minutes with 20 c.c. of Fehling's solution. Pellet suggests, therefore, the following process of estimating small quantities of reducing sugar in presence of large quantities of sucrose.

**Pellet's Method.**—The Fehling's solution used is prepared as follows:

Solution A. 34.64 grm. of copper sulphate made up to 500 c.c.

Solution B. Rochelle salt, 180 grm.  
Sodium hydroxide (sticks), 60 grm.  
Water to make to 500 c.c.

This solution, it may be noted, is very nearly the same in composition as that adopted by Brown, Morris and Millar (page 397). 10 c.c. of the mixed solution corresponds with 0.050 grm. of invert sugar.

To estimate the quantity of reducing sugar in highly purified and half-purified cane and beet products, 25 grm. of the material are dissolved in 100 c.c. of water; 20 c.c. of this solution is mixed with 20 c.c. of the Fehling's solution and 10 c.c. of water and the mixture heated in a beaker flask (see page 398) during 10 minutes in a water-bath at 60–62°. 50 c.c. of cold water are then added and the cuprous oxide collected at once, thoroughly washed and ignited to cupric oxide. Pellet makes use of a filter paper to collect the precipitate, in which case a blank experiment has to be made, with the same volume of Fehling's solution, in order to ascertain how much copper is taken up by the filter paper itself under exactly similar conditions. Pellet suggests using small filter papers having a diameter of about 3 cm. in conjunction with a special filtering arrangement devised for the purpose. With these small discs of filter paper the method can be used colorimetrically, the proportion of cuprous oxide being judged by the depth of colour on the disc of filter paper of known size; for this purpose a series of standards are prepared by means of known very small quantities of invert sugar. Under the conditions Pellet used, the weight of reducing sugar (invert sugar) is calculated by multiplying the weight of cupric oxide ( $\text{CuO}$ ) by 0.453. But the value of the constant will vary slightly according to the dimensions of the vessel in which the mixture of sugar and Fehling's solution is heated, that is, according to the proportion of the free surface to the depth, so that each worker should ascertain for himself the value of this ratio for his own conditions, by using a dilute solution of invert sugar (prepared from pure sucrose) of known concentration.

In the above-described methods, the precipitated cuprous oxide is either weighed as such or after conversion into cupric oxide or metallic copper. If desired, the cuprous oxide may be treated according to one of the following methods, all of which are approved as official methods by the A. O. A. C.

### 1. Reduction in Hydrogen

Filter the cuprous oxide immediately through a weighed filtering tube made of hard glass, using suction. Support the asbestos film in the filtering tube with a perforated disc or cone of platinum and

wash free from loose fibres before weighing; moisten previous to the filtration. Provide the tube with a detachable funnel during the filtration, so that none of the precipitate accumulates near the top, where it could be removed by the cork used during the reduction of the cuprous oxide. Transfer all the precipitate to the filter and thoroughly wash with hot water, following the water by alcohol and ether successively. After being dried, connect the tube with an apparatus for supplying a continuous current of dry hydrogen, gently heat until the cuprous oxide is completely reduced to the metallic state, cool in the current of hydrogen, and weigh. If preferred, a Gooch crucible may be used for the filtration.

## **2. Electrolytic Deposition from Sulphuric Acid Solution**

Filter the cuprous oxide in a Gooch crucible, wash the beaker and precipitate thoroughly with hot water without any effort to transfer the precipitate to the filter. Wash the asbestos film and the adhering cuprous oxide into the beaker by means of hot dilute nitric acid. After the copper is all in solution, re-filter through a Gooch crucible with a thin film of asbestos and wash thoroughly with hot water. Add 10 c.c. of dilute sulphuric acid, containing 200 c.c. of sulphuric acid (sp. gr. 1.84) in 1000 c.c., and evaporate the filtrate on the steam-bath until the copper salt has largely crystallised. Heat carefully on a hot plate or over a piece of asbestos board until the evolution of white fumes shows that the excess of nitric acid is removed. Add from 8 to 10 drops of nitric acid (sp. gr. 1.42) and rinse into a platinum dish of from 100 to 125 c.c. capacity. Precipitate the copper by electrolysis. Wash thoroughly with water before breaking the current, remove the dish from the circuit, wash with alcohol and ether successively, dry at about 50° and weigh. If preferred, the electrolysis can be conducted in a beaker, the copper being deposited upon a weighed platinum cylinder.

## **3. Electrolytic Deposition from Sulphuric and Nitric Acid Solution**

Filter and wash as under (2). Transfer the asbestos film from the crucible to the beaker by means of a glass rod and rinse the crucible with about 30 c.c. of a boiling mixture of dilute sulphuric and nitric acids, containing 65 c.c. of sulphuric acid (sp. gr. 1.84) and 50 c.c. of nitric acid (sp. gr. 1.42) in 1000 c.c. Heat and agitate until solution is complete; filter and electrolyse as under (2).

#### 4. Electrolytic Deposition from Nitric Acid Solution

Filter and wash as under (2). Transfer the asbestos film and adhering oxide to the beaker. Dissolve the oxide still remaining in the crucible by means of 2 c.c. of nitric acid (sp. gr. 1.42), adding it with a pipette and receiving the solution in the beaker containing the asbestos film. Rinse the crucible with a jet of water, allow the rinsings to flow into the beaker. Heat the contents of the beaker until the copper is all in solution, filter, dilute the filtrate to a volume of 100 c.c. or more, and electrolyse. When a nitrate solution is electrolysed, the first washing of the deposit should be made with water acidified with sulphuric acid, in order that the nitric acid may be all removed before the current is interrupted.

#### 5. Volumetric Permanganate Method

Filter and wash the cuprous oxide as described for method (2). Transfer the asbestos film to the beaker, add about 30 c.c. of hot water, and heat the precipitate and asbestos thoroughly. Rinse the crucible with 50 c.c. of a hot saturated solution of ferric sulphate in 20% sulphuric acid, receiving the rinsings in the beaker containing the precipitate. After the cuprous oxide is dissolved, wash the solution into a large Erlenmeyer flask and immediately titrate with a standard solution of potassium permanganate. 1 c.c. of the permanganate solution should equal 0.010 gm. of copper. In order to determine the strength of this solution, make 6 or more determinations with the same sugar solution, titrating one-half of the precipitate obtained, and determining the copper in the others by electrolysis. The average weight of copper obtained by electrolysis, divided by the average number of c.c. of permanganate solution required for the titration is equal to the weight of copper equivalent to 1 c.c. of the standard permanganate solution. A solution standardised with iron or oxalic acid will give too low results.

#### 6. Low's Volumetric Method, Modified<sup>1</sup>

##### (a) STANDARDISATION OF THE THIOSULPHATE SOLUTION

Prepare a solution of sodium thiosulphate containing 19 grms. of pure crystals to 1000 c.c. Weigh accurately about 0.2 gm. of pure

<sup>1</sup>*J. Amer. Chem. Soc.*, 1902, 24, 1082.

copper foil and place in a flask of 250 c.c. capacity. Dissolve by warming with 5 c.c. of a mixture of equal volumes of strong nitric acid and water. Dilute to 50 c.c., boil to expel the red fumes, add 5 c.c. of strong bromine water, and boil until the bromine is thoroughly expelled. Remove from the heat and add a slight excess of strong ammonium hydroxide—7 c.c. is about the right amount. Again boil until the excess of ammonia is expelled, as shown by a change of colour of the liquid and a partial precipitation. Now add a slight excess of strong acetic acid (3 or 4 c.c. of 80% acid) and boil for a minute. Cool to room temperature and add 10 c.c. of a solution of pure potassium iodide containing 300 grm. of potassium iodide to 1000 c.c. Titrate at once with the thiosulphate solution until the brown tinge has become weak, then add sufficient starch liquor to produce a marked blue colouration. Continue the titration cautiously until the colour due to free iodine has entirely vanished. The blue colour changes toward the end to a faint lilac. If at this point the thiosulphate be added drop by drop and a little time be allowed for complete reaction after each addition, there is no difficulty in determining the end point within a single drop. 1 c.c. of the thiosulphate solution will be found to correspond to about 0.005 grm. of copper.

(b) DETERMINATION OF COPPER

After washing the precipitated cuprous oxide, cover the Gooch crucible with a watch glass and dissolve the oxide by means of 5 c.c. of warm nitric acid (1 : 1) poured under the watch glass with a pipette. Catch the filtrate in a flask of 250 c.c. capacity, wash the watch glass and Gooch crucible free of copper; 50 c.c. of water will be sufficient. Boil to expel red fumes, add 5 c.c. of bromine water, boil off the bromine, and proceed exactly as in standardising the thiosulphate.

**Volumetric Estimation of Reducing Sugars by Fehling's Solution.** The results obtained by using Fehling's solution volumetrically are not generally so accurate as those given by the gravimetric method. The operation should be *quickly* conducted.

In all cases in which Fehling's solution is to be used volumetrically its true oxidising power under the conditions of the experiment should be ascertained by actual trial.

The volumetric methods adopted by the A. O. A. C. as tentative methods are as follows:

**(a) Applicable to Invert Sugar and Dextrose**

Place 10 c.c. of the mixed copper reagent in a large test-tube and add 10 c.c. of distilled water. Heat to boiling, and gradually add small portions of the solution of the material to be tested until the copper has been completely precipitated, boiling to complete the reaction after each addition. Two minutes' boiling is required for complete precipitation when the full amount of sugar solution has been added in one portion. When the end reaction is nearly reached and the amount of sugar solution to be added can no longer be judged by the colour of the solution, remove a small portion of the liquid and filter rapidly into a small porcelain crucible or on a test plate; acidify with dilute acetic acid, and test for copper with a dilute solution of potassium ferrocyanide. The sugar solution should be of such strength as will give a burette reading of 15 to 20 c.c. and the number of successive additions should be as small as possible.

Since the factor of calculation varies with the minute details of manipulation, every operator must determine a factor for himself, using a known solution of a pure sample of the sugar which he desires to determine, and keeping the conditions the same as those used for the determinations.

Standardise the solution for invert sugar in the following manner:

Dissolve 4.75 grm. of pure sucrose in 75 c.c. of water, add 5 c.c. of hydrochloric acid (sp. gr. 1.188), and invert as under the official method for sucrose, page 378. Neutralise the acid exactly with sodium hydroxide and dilute to 1000 c.c. 10 c.c. of this solution contain 0.050 grm. of invert sugar, which should reduce 10 c.c. of the copper solution; the copper solution should never be taken as a standard, but should be checked against the sugar. In case this method is used for determining dextrose, pure dextrose must be used in standardising the solution.

**(b) Soxhlet's Method**

Make a preliminary titration to estimate the approximate percentage of reducing sugar in the material under examination. Pre-

pare a solution which contains approximately 1% of reducing sugar. Place in a beaker 100 c.c. of the mixed copper reagent and approximately the amount of the sugar solution for its complete reduction. Boil for 2 minutes. Filter through a folded filter and test a portion of the filtrate for copper by use of acetic acid and potassium ferrocyanide. Repeat the test, changing the volume of sugar solution, until two successive amounts are found which differ by 0.1 c.c., one giving complete reduction and the other leaving a small amount of copper in solution. The mean of these two readings is taken as the volume of the solution required for the complete precipitation of 100 c.c. of the copper reagent.

Under these conditions 100 c.c. of the mixed copper reagent require 0.475 grm. of anhydrous dextrose or 0.494 grm. of invert sugar for complete reduction. Calculate the percentage by the following formula:

$V$  = the volume of the sugar solution required for the complete reduction of 100 c.c. of the copper reagent.

$W$  = the weight of the sample in 1 c.c. of the sugar solution.

Then  $\frac{100}{V} \times 0.475 =$  per cent. of dextrose,

or  $\frac{100}{V} \times 0.494 =$  per cent. of invert sugar.

The titration of raw sugars, malt worts and other coloured commercial products with Fehling's solution, employing potassium ferrocyanide as indicator, is often anything but an accurate method and is very tedious. When certain amino-compounds are present so much cuprous oxide may be dissolved that it is impossible to obtain an acidified filtrate which gives no colour with potassium ferrocyanide. Indicators, which respond to a minute trace of cupric salt and can be employed without filtering off a portion of the liquid, have been proposed. E. F. Harrison (*Pharm. J.*, 1903, 71, 170) uses a solution of starch and potassium iodide which when acidified with acetic acid and brought into contact with a cupric salt liberates iodine and blue is developed.

Still more satisfactory is a solution of ferrous thiocyanate as suggested by A. R. Ling, T. Rendle and G. C. Jones (*Analyst*, 1905,

30, 182; 1908, 33, 160). When a drop of this on a white slab is brought into contact with a drop of a solution of a cupric salt the characteristic red colour of ferric thiocyanate is produced. Ling's method has been adopted by the Malt Analysis Committee of the Institute of Brewing (*J. Inst. Brewing*, 1906, 12, 1) and is given below:

**Preparation of the Indicator.**—1 grm. of ferrous ammonium sulphate and 1.5 grm. of ammonium thiocyanate are dissolved in 10 c.c. of water at a moderate temperature, say at 45–50°, and immediately cooled; 2.5 c.c. of concentrated hydrochloric acid are then added. The solution so obtained is invariably brownish-red, due to the presence of ferric salt, which latter must be reduced. For this purpose, zinc dust is the most satisfactory reagent, and a mere trace is sufficient to decolorise the solution if pure reagents have been employed.

When kept for some hours, the indicator re-develops the red by oxidation. It may, however, be decolorised by the addition of a further quantity of zinc dust, but its delicacy is decreased after it has been decolourised several times. For practical purposes the indicator may be too delicate and it is recommended to prepare it the day before it is required for use, as it gives the best results after the second decolorisation. Although the thiocyanate indicator is the most sensitive indicator yet devised, it cannot be used with commercial products containing iron, and in these cases the ferrocyanide indicator should be employed. The Fehling's solution is prepared as follows:

**Solution No. 1.**—Crystallised copper sulphate (69.278 grm.) is dissolved in water and the solution made up to 1 litre.

**Solution No. 2.**—Crystallised Rochelle salt (346 grm.) is dissolved in hot water and mixed with sodium hydroxide (142 grm.) also dissolved in water. After cooling the mixed solutions are made up to 1 litre.

Equal volumes of these two solutions are accurately measured out at 15.5° (or any other standard temperature adopted) and this mixture constitutes the reagent. It is standardised as follows: pure sucrose (0.95 grm.) is dissolved in water (150 c.c.), and the solution boiled with 30 c.c. of N/2 hydrochloric acid for exactly 1 minute; the solution is cooled, neutralised by adding N/2 sodium hydroxide (30 c.c.) and then diluted to 500 c.c. This solution, which

contains 0.2 grm. of invert sugar per 100 c.c., is titrated against 10 c.c. portions of the copper reagent.

The method of titration is as follows: Freshly mixed Fehling's solution (10 c.c.) is accurately measured into a 200 c.c. boiling flask and raised to boiling. The sugar solution, which should be adjusted to such a strength that 20 to 30 c.c. of it are required to reduce 10 c.c. of Fehling's solution, is then run into the boiling liquid in small amounts, commencing with 5 c.c. After each addition of sugar solution, the mixture is boiled, the liquid being kept rotated. About a dozen drops of the indicator are placed on a porcelain or opal glass slab and when it is judged that the precipitation of cuprous oxide is complete, a drop of the liquid is withdrawn by a clean glass rod or by a capillary tube and brought in contact with the middle of a drop of the indicator on the slab. The test must be carried out rapidly. It is also essential to perform the titration as rapidly as possible, as an atmosphere of steam is then kept in the neck of the flask and the influence of atmospheric oxygen is avoided. At the final point the liquid is boiled for about 10 seconds. As in the ordinary volumetric method, the first titration may only give approximate results and a second or third will then be necessary to establish the end-point accurately. However, when the operator has gained experience, the first titration is as much to be relied on as succeeding ones. One titration takes about 3 minutes. The authors claim the average error of the method to be about 1 in 300.

The amount of reducing sugar is found from the table of Ling and Jones (see below).

The manner of using this table is best explained by an illustration. Suppose a solution of pure lævulose is being examined and that 25 c.c. of it are required to reduce 10 c.c. of Fehling's solution. Opposite 25 in the first column is found 0.2138 in column *L*; the percentage of lævulose in the solution titrated is thus given direct. If 25.2 c.c. or other quantity not a whole number is required to reduce 10 c.c. of Fehling's solution, the percentage of lævulose can easily be found by interpolation between the numbers given in column *L*.

The table also gives the number of c.c. of Fehling's solution equivalent to 1 grm. of the particular sugar at each concentration. The numbers given in columns *D'* *L'*, *I'* and *M'* are used for another purpose—namely the separate determination of two reducing sugars

in a mixture by a modification of Morris' method (*J. Inst. Brewing*, 1898, 4, 162) (see page 192).

TABLE OF REDUCING POWER OF DIFFERENT SUGARS  
ACCORDING TO LING AND JONES

Volume of solution required by 10 c.c. Fehling's solution, c.c.	Dextrose		Lævulose		Invert sugar		Maltose	
	D Dex- trose in 100 c.c. of solution, gram.	D' Fehling's solution equiva- lent to 1 gram. dex- trose, c.c.	L Lævu- lose in 100 c.c. of solution, gram.	L' Fehling's solution equiva- lent to 1 gram. lævu- lose, c.c.	I Invert in 100 c.c. of solution, gram.	I' Fehling's solution equiva- lent to 1 gram. invert, c.c.	M Mal- tose in 100 c.c. of solution, gram.	M' Fehling's solution equiva- lent to 1 gram. maltose, c.c.
20	0.2427	206.0						
21	0.2332	205.1	.....	.....	0.2412	197.5	0.3888	122.5
22	0.2226	204.2	0.2411	188.5	0.2311	196.8	0.3711	
23	0.2138	203.4	0.2312	188.0	0.2218	196.0	0.3550	
24	0.2056	202.6	0.2222	187.5	0.2132	195.5	0.3402	
25	0.1981	201.9	0.2138	187.1	0.2052	194.9	0.3265	
26	0.1911	201.3	0.2060	186.7	0.1980	194.3	0.3140	
27	0.1846	200.7	0.1988	186.3	0.1910	193.9	0.3023	
28	0.1784	200.1	0.1921	186.0	0.1846	193.4	0.2915	
29	0.1728	199.6	0.1857	185.6	0.1787	193.0	0.2815	
30	0.1675	199.1	0.1798	185.4	0.1731	192.5	0.2721	
31	0.1625	198.6	0.1743	185.1	0.1678	192.2	0.2633	
32	0.1577	198.2	0.1691	184.8	0.1629	191.8	0.2551	
33	0.1532	197.8	0.1642	184.6	0.1583	191.5	0.2474	
34	0.1490	197.4	0.1596	184.3	0.1539	191.2	0.2401	
35	0.1450	197.0	0.1552	184.1	0.1497	190.9	0.2332	
36	0.1412	196.7	0.1511	183.9	0.1458	190.6	0.2268	
37	0.1377	196.4	0.1472	183.6	0.1421	190.3	0.2206	
38	0.1343	196.0	0.1435	183.4	0.1385	190.1	0.2148	
39	0.1310	195.8	0.1399	183.3	0.1349	189.8	0.2093	
40	0.1279	195.5	0.1366	183.1	0.1319	189.6	0.2041	122.5
41	.....	.....	0.1334	182.9	0.1288	189.4		
42	.....	.....	0.1298	182.8	0.1259	189.2		
43	.....	.....	0.1274	182.6				

**Estimation of Invert Sugar in Presence of Sucrose.**—Ling and Rendle (*Analyst*, 1908, 33, 170) have determined the corrections to be applied in order to allow for the influence of the sucrose when present in mixtures of sugars. In the following table:

Column *A* gives the amount in grams of sucrose present in 100 c.c. of the sugar solution.

Column *B* gives the percentage of sucrose present expressed on the total sugars.

Column *C* gives the percentage of invert sugar present expressed on the total sugars.

Column *D* gives the number of c.c. of sugar solution required to reduce 10 c.c. of Fehling's solution.

Column *E* gives the percentage of invert sugar on the total sugars found by direct experiment.

Column *F* gives the differences between the values shown in columns *C* and *E*.

The influence of the sucrose is seen to be practically negligible until the proportion to the total sugars (sucrose + invert sugar) amounts to 30%, at which point the invert sugar is overestimated by 0.2%. The influence of the sucrose increases progressively until the propor-

EACH SOLUTION CONTAINED IN ADDITION TO THE SUCROSE  
SHOWN UNDER COLUMN *A*, 0.2 GRM. OF INVERT SUGAR  
PER 100 C.C.

<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>
0.01	4 8	95 2	25 60	95 30	0.10
0.03	13 0	87 0	25 60	87 10	0.10
0.05	20 0	80 0	25 60	80.10	0.10
0.10	33 3	66 7	25 55	66 90	0.20
0.20	50 0	50 0	25 45	50 40	0.40
0.30	60 0	40 0	25 40	40 40	0.40
0.40	66 6	33 4	25 35	33 80	0.40
0.50	71.4	38 6	25 30	29 00	0.40
0.60	75 0	25 0	25 20	25 40	0.40
0.70	77.7	22 3	25 15	22.70	0.40
0.80	80 0	20 0	25 10	20 40	0.40
1.25	86 2	13 8	25 05	14 10	0.30
1.50	88 2	11 8	24 95	12 10	0.30
1.75	89 7	10 3	24 85	10 60	0.30
1.75	89 7	10 3	24 80	10 60	0.30
2.00	90 9	9 1	24 70	9 45	0.35
2.00	90 9	9 1	24 80	9 41	0.31
2.50	92 5	7 5	24 80	7 76	0.26
3.00	93 8	6 2	24 70	6 44	0.24
5.00	96 1	3 9	24 20	4 05	0.15
7.00	97 2	2 8	23 60	3 04	0.24
10.00	98 0	2 0	22 95	2 23	0.23
20.00	99 0	1 0	22 40	1 14	0.14
25.00	99 2	0 8	22 25	0 92	0.12
30.00	99 3	0 7	22 25	0 80	0.10

tion of the latter expressed on the total sugars is 99.3%, beyond which point it was not determined. At this point the invert sugar is overestimated by about 15%. It must be remembered that the percentages of invert sugar decrease as the percentage of sucrose increases, and the correction to be applied (column *F*) is in concrete numbers greatest when the percentage of sucrose in the total sugars is between 50 and 80%. In the case of a mixture of equal parts of sucrose and invert sugar the latter would be estimated, if no correction were made, as 50.4 instead of 50%, whilst in the case of a mixture of 99 parts of sucrose and 1 of invert sugar the latter would be estimated as 1.14% instead of 1%. Ling generally deducts the

values shown in column *F* from the values ascertained by direct titration of the mixture of sugars. For this purpose it is necessary to know the percentage of sucrose not calculated on the sample, but on the total sugars (sucrose + invert sugar) in the sample. This can be estimated by the Clerget method or by the method of double titration before and after hydrolysis with hydrochloric acid, applying the formula  $S = \frac{95I' - I}{100}$  in which *S* is the approximate percentage of sucrose, *I* the apparent percentage of invert sugar, *i. e.*, the value obtained by direct titration, and *I'* is the percentage of invert sugar obtained by titration after complete hydrolysis.

The approximate value given in column *F* is subtracted from the value of *I* and added to the value of *S*, the respective results giving the corrected percentages of invert sugar and of sucrose. In order to express these on the sample, each of the values is multiplied by  $S + I/100$ . To be exact, the value added to the approximate percentage of sucrose, *S*, should be diminished by 5%, but the degree of accuracy of the method does not warrant this refinement, seeing that the corrections to be applied are comparatively small.

**Titration with Pavy's Ammoniacal Cupric Solution.**—This modification of the ordinary mode of using Fehling's solution for the estimation of reducing sugars is based on the fact that in the presence of a sufficient excess of ammonium hydroxide the cuprous oxide is not precipitated, but forms a *colourless* solution, so that the end of the reaction is indicated by the decolorisation of the blue liquid. As the ammoniacal cuprous solution is extremely oxidisable, the blue colour being restored by oxidation, it is necessary to avoid access of air. This is best done by attaching the tip of the Mohr's burette containing the sugar solution to a tube passing through the rubber stopper of a flask containing the copper solution. A second tube conveys the steam and ammoniacal gas into a flask of cold water. It is desirable to allow the end of the tube to dip into a little mercury placed at the bottom of the water, so as to prevent any tendency to "suck back." A still better arrangement is to pass (by a third tube) a slow current of hydrogen or illuminating gas through the flask containing the boiling copper solution.

To prepare the ammoniacal solution, 120 c.c. of the ordinary Fehling's solution (see page 392) should be mixed with 300 c.c. of strong aqueous ammonia (sp. gr. 0.880), and with 400 c.c. of sodium hydrox-

ide solution of 1.14 sp. gr. (= 12%). The mixture is then made up to 1 litre. 100 c.c. of this solution has the same oxidising power on dextrose as 10 c.c. of the ordinary Fehling's solution, that is, it corresponds to 0.050 grm.

In carrying out the process, 100 c.c. of the above solution are placed in the flask, a few fragments of pumice or pipe-stem added, the tubes and burette adjusted, and the liquid brought to boiling. The sugar solution is then gradually run in from the burette, the boiling being continued regularly. The process is at an end when the blue colour of the liquid is wholly destroyed. The end-reaction is very sharply marked, but the reduction occurs more slowly than with the ordinary Fehling's solution. The process is often a very useful one, especially for the rapid analysis of impure saccharine liquids, such as beer-worts.

O. Hehner has shown (*Analyst*, 1881, 6, 218) that the presence of alkaline tartrates and carbonates gravely affects the accuracy of indications obtained with Pavy's solution.

Pavy's solution has been very largely used in clinical chemistry, in particular in urine analysis. It has, however, many practical disadvantages, *e. g.*, the inconvenience of working with an ammoniacal solution and the great dilution as compared with the ordinary Fehling's solution. Its application, though giving very trustworthy results in experienced hands, should be practised with caution.

Pavy's solution is of the greatest service in such cases as studied by Croft Hill (*Trans. Chem. Soc.*, 1898, 73, 634) and E. F. Armstrong, in which it was required to ascertain very accurately proportions of maltose and dextrose in solutions, the total sugar-content of which, remained constant.

Blythe ascertains the end reaction more accurately by bubbling air through the liquid directly the decolorisation is complete. The blue colour should reappear after a very few seconds unless too much sugar solution has been added when a longer time elapses.

Pavy's solution possesses a different oxidising power on maltose and lactose from that exerted by Fehling's solution. Its reaction on invert sugar is, under the above-described conditions, only five-sixths of that exerted by Fehling's solution. Hence 120 c.c. of the latter are employed in making the ammoniacal solution, instead of 100, as would be the case if they were strictly equivalent.

**Barfoed's reagent** is prepared by dissolving 13.3 grm. of crystallised neutral copper acetate in 200 c.c. of 1% acetic acid (*Zeitsch. anal. Chem.*, 1873, 12, 27). It forms a delicate test for dextrose and, moreover, is not reduced by either maltose or lactose under certain conditions—*i. e.*, less than 2 minutes' heating. More prolonged heating will cause hydrolysis of the di-saccharides and reduction. The method has been applied quantitatively, but its indications are unreliable.

The behaviour of a modified form of the reagent with dextrose, maltose, lactose and sucrose has been investigated by Hinkel and Sherman (*J. Amer. Chem. Soc.*, 1907, 29, 1744) who claim that by using 5 c.c. of the reagent the presence of 0.0004 grm. dextrose can be shown, either alone or mixed with di-saccharides, provided that the total weight of disaccharide does not exceed 0.02 grm. The test is best made by heating in test-tubes in a boiling water-bath for 3 minutes. Under these conditions lactose or maltose does not cause reduction until heated for 9 or 10 minutes. Roaf (*Biochem. J.*, 1908, 3, 182) has made use of the method to demonstrate the hydrolysis of lactose and maltose by enzymes.

**Reaction of Sugars with Mercury Solutions.**—Several methods have been described of estimating reducing sugars by their action on mercuric solutions, an alkaline solution of potassium mercuric cyanide being recommended by Knapp; an alkaline solution of potassium mercuric iodide by Sachsse; and a solution of mercuric acetate by Hager. The first two of these reagents have valuable qualities. They cannot advantageously replace that of Fehling for ordinary purposes, but may occasionally be applied with advantage, being unequally affected by the different kinds of reducing sugars. Their use is also open to the disadvantage that mercury solutions are likewise reduced by creatine, creatinine, glycerol and, in some cases, even by alcohol (Guillaume, Gentil, *Compt. rend.*, 1881, 93b, 338).

**Knapp's mercuric solution** (*Zeitsch. anal. Chem.*, 1870, 9, 395) is prepared by dissolving 10 grm. of pure dry mercuric cyanide in water, adding 100 c.c. of sodium hydroxide solution of 1.145 sp. gr. and diluting the liquid to 1 litre.

40 c.c. of the reagent are diluted to 100 c.c., heated to boiling and the sugar solution, not stronger than 0.5%, is run in *as quickly as possible* until the whole of the mercury is precipitated. To determine this point a strip of filter-paper is moistened with the clear

liquid and tested with hydrochloric acid and hydrogen sulphide for mercury. This method has been carefully investigated by Otto (*J. prakt. Chem.*, 1882, 26, 87).

**Sachsse's mercuric solution** (*Zeitsch. Ver. deut. Zuckerind.*, 1876, 26, 872) is prepared by dissolving 18 gm. of pure dry mercuric iodide in a solution of 25 gm. of potassium iodide. To this, a solution of 80 gm. of potassium hydroxide is added, and the solution diluted to 1 litre. 40 c.c. of this solution are boiled in a basin, and a standard solution of the sugar gradually added. The end of the reaction is attained when a drop of the supernatant liquid ceases to give a brown colour with a drop of a very alkaline solution of stannous chloride. The end of the reaction is well defined, and the results are accurate when pure dextrose or invert sugar is worked with, though differing with each. In presence of sucrose the results are quite erroneous. By reducing the proportion of potassium hydroxide from 80 gm. to 10 gm. per litre Heinrich finds that dextrose may be accurately estimated in presence of very varying amounts of sucrose.

Soxhlet has shown that less dextrose is required the more slowly it is added and that the concentration is of considerable influence. 100 c.c. of the solution require 0.325 gm. dextrose in 0.5% and 0.330 gm. in 1% solution. 1 gm. dextrose in 1% solution reduces 302.5 c.c. Sachsse's solution.

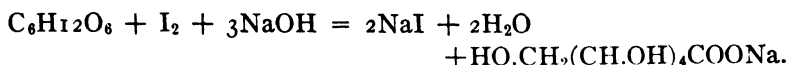
For information respecting other modifications of these methods Lippmann's *Chemie der Zuckerarten* should be consulted.

## METHOD OF ANALYSIS DEPENDING ON OXIDATION WITH IODINE

Various procedures for estimating dextrose and other aldose sugars by oxidation with iodine in alkaline solution have been proposed from time to time. The reaction consists in the oxidation of the aldose into the corresponding acid, dextrose, for example, being converted into gluconic acid. The reaction is not strictly stoichiometric, however, and the process is further complicated by the fact that sugars, other than aldoses, react slightly with iodine.

The procedure devised by Willstätter and Schudel (*Ber.*, 1918, 51, 780) is as follows: The solution of aldose is mixed with about

twice the amount of N/10 iodine solution necessary for oxidation, a quantity of N/10 sodium hydroxide, which is 1.5 times as much as the iodine, then being slowly added. The mixture is allowed to stand for 12 to 15 minutes (or 20 minutes if the proportion of sugar is very small) and then acidified slightly with sulphuric acid, the excess of iodine being titrated with N/10 thiosulphate.



The above procedure has been examined by H. M. Judd (*Biochem. J.*, 1920, **14**, 255) and by Baker and Hulton (*Biochem. J.*, 1920, **14**, 754) who find that a period of 3 to 5 minutes suffices for complete oxidation of the sugar and that the relations between the various sugars and iodine are as follows:

1 gm. of dextrose	= 1.41 gm. of iodine
1 gm. of lævulose	= 0.10 gm. of iodine
1 gm. of galactose	= 1.37 gm. of iodine
1 gm. of sucrose	= 0.02 gm. of iodine
1 gm. of maltose	= 0.746 gm. of iodine
1 gm. of lactose	= 0.762 gm. of iodine
1 gm. of raffinose	= 0.05 gm. of iodine
1 gm. of soluble starch	= 0.06 gm. of iodine

Taken in conjunction with other methods, the iodometric method is of considerable value in the analysis of mixtures of pure sugars or of commercial products, such as invert sugar or honey, which contain very little organic non-sugar. The method cannot be applied to materials such as molasses, however, which contain considerable proportions of organic non-sugar substances, since the latter may absorb a considerable amount of iodine.

## METHODS OF ANALYSIS DEPENDING ON FERMENTATION

**Fermentation of Sugars.**—Dextrose, lævulose, mannose, and invert sugar are fermentable by all yeasts.<sup>1</sup> Sucrose, maltose, lactose, melibiose and raffinose are fermentable only after inversion by dilute acids or by an appropriate enzyme. Ordinary yeast, *Saccharomyces cerevisiæ*, contains the enzymes which hydrolyse sucrose and

<sup>1</sup> See E. F. Armstrong, *Proc. Roy. Soc.*, 1905, **76B**, 600.

maltose and can ferment both these sugars. Fermentation is preceded by inversion and, indeed, if the proportion of yeast is very small, the change of sucrose does not go beyond the formation of invert sugar.

To recognise the presence of a fermentable sugar by means of yeast, care must be taken (1) that the aqueous solution is not too concentrated (from 5 to 10% is the most suitable concentration); (2) that the liquid is neutral or faintly acid, alkalinity being carefully neutralised; (3) that the liquid is wholly free from antiseptics of any kind which would prevent the alcoholic fermentation.

The yeast should be fresh, free from starch and carefully washed with a little cold, distilled water before use.

5 to 10 c.c. of the liquid are mixed with a little yeast, placed in a test-tube closed with a plug of sterilised cotton wool, and incubated at 20–30° for a few hours. In a positive experiment the signs of fermentation are unmistakable, and gentle shaking will cause the bubbles of gas to rise to the surface. It is always desirable to make a blank experiment so as to ascertain positively that the yeast does not itself yield any notable quantity of carbon dioxide under the conditions of the experiment.

The foregoing process may readily be made roughly quantitative by attaching a delivery tube and collecting the gas formed over mercury. More accurate results are obtained by using an apparatus such as is employed for the analysis of carbonates and determining the loss of weight during fermentation. The dissolved carbon dioxide may be swept out by a current of air before the final weighing. The fermentation should be continued as long as any notable quantity of gas continues to be evolved. The weight of carbon dioxide multiplied by 2.0454 gives that of the invert sugar or dextrose fermented, which figure multiplied by 0.95 gives the corresponding weight of sucrose or maltose.

Instead of measuring or weighing the carbon dioxide produced it is in some respects preferable to estimate the *alcohol* formed. The process is conducted as already described, but it is not desirable to employ less than 50 to 100 c.c. of the solution, which should by preference have a concentration of 12 to 16%; 0.5 grm. of pressed fresh yeast is sufficient in most cases, especially if a little yeast-ash be added, but it is desirable to add a little more yeast at the end of the action to insure that no further fermentation can be induced. The

liquid should be kept at a temperature of  $20^{\circ}$  to  $25^{\circ}$  for 2 or 3 days, after which the liquid is distilled to about one-third, the distillate weighed, and the alcohol contained in it ascertained from the sp. gr. The weight of alcohol thus found, when multiplied by 2.02, gives the invert sugar or by 1.96 the sucrose from which it was derived.

Some operators prefer to employ a large quantity of yeast, such as 10 or even 20 grm. In such cases it is very desirable to conduct a blank experiment with the same quantity of yeast and water, side by side with the test of the saccharine liquid, and to deduct the alcohol found in the former case from that obtained in the latter before calculating to the equivalent of sugar. A still better plan, perhaps, is to ferment a solution of sucrose or invert sugar, of known strength, side by side with the samples, when the amounts of sugar in the two liquids will bear to each other the same proportion as the amounts of alcohol produced by their fermentation.

Another method which has been suggested for estimating sugar from the results of its fermentation by yeast consists in noting the "gravity lost" in the process; that is, the sp. gr. of the original saccharine solution is observed and compared with that of the fermented liquid, after filtering, washing the residue, boiling off the alcohol, and making up the solution to its original volume. The difference is the "gravity lost" by the fermentation. The "spirit indication" corresponding to the value thus found is ascertained by reference to the table on page 205, and this figure subtracted from 1000 gives the density of the dilute alcohol produced by the fermentation. The strength of this can be ascertained by reference to the tables, and the weight so arrived at can be calculated into its equivalent of sucrose or maltose by means of the factor 1.96, or into dextrose by the factor 2.02. The dextrose may also be deduced by calculating 0.219% for each degree of gravity lost.

It is evident that the last-described method can be advantageously employed as a check on the distillation process.

Instead of estimating the sugar from the sp. gr. of the solution before and after fermentation, equal volumes of the original and the filtered fermented liquids may be evaporated to dryness, and the quantity of sugar deduced from the loss of weight. An addition of 5% to the amount of sugar thus found should be made as a correction for the succinic acid and glycerol which are produced by the fermentation and remain in the residue from the fermented liquid. When

the quantity of sugar is small, this method is preferable to an estimate based on the gravity lost.

In estimating sugar by fermentation with yeast, it is desirable to add to the solution a little yeast-ash or sodium phosphate and potassium nitrate, so as to furnish the yeast with the inorganic elements requisite for its nutrition.

The estimation of sugar by fermentation with yeast is occasionally very valuable, and when the process is carefully conducted the results are fairly accurate.

The quantitative fermentation of sugars requires from 6 to 7 days or longer if maltose is to be fermented in presence of dextrin. The use of yeast affords practically the only accurate method of separating dextrose from maltose in the analysis of starch syrups.

The application of pure culture yeasts to the separation of different sugars promises valuable results. The sugar not attacked by the yeast in a mixture of fermentable and unfermentable carbohydrates can be estimated accurately; the estimation of the quantity of the fermented sugar from the carbon dioxide lost does not always give trustworthy results.

Kluyver has applied somewhat similar principles, with considerable success, to the estimation of the different constituent sugars present in complex mixtures of sugars, for example of maltose, sucrose, raffinose, with the simple hexoses. Full details are given in the monograph *Biochemische Suikerbepalingen* (Leiden, E. J. Brill, 1914). Kluyver makes use of a gasometric method, measuring the volume of carbon dioxide evolved by the action of a series of special yeasts and torulæ upon the solution dealt with; this method has the advantage that approximately accurate results are obtained with exceedingly small quantities of the solutions or sugar dealt with. A special eudiometer was devised in which the fermentation is carried out and the gas measured. A special case may be cited to illustrate the principle of applying this process, the sugars present, for example, being raffinose, sucrose, and monoses; the ferments used were *S. cerevisiæ* (under-yeast *U*), ordinary press yeast, *Torula dattila* and *Torula monosa*. The difference between the results with the first two yeasts gives the raffinose; the sucrose can then be calculated from the volume of gas given by *Torula dattila*, after allowing for the raffinose present, whilst the gas evolved

by *Torula monosa* is a measure of the hexoses present (dextrose, lævulose or mannose), as only these sugars are fermented by this organism. The process is said to be relatively rapid and to give approximately accurate results in cases where other methods are inapplicable. Biochemical methods have been applied by Kluyver to the analysis of food materials such as jams, potato syrups and to other cases of technical importance.

The experiments of Bertrand and Weisweiler and Bertrand and Ducháček (*Ann. Inst. Pasteur*, 1909, **23**, 402) have shown that the Bulgarian bacillus (*Bacillus acidi lactici* Massol) is capable of converting hexoses such as dextrose, lævulose, mannose and galactose into lactic acid, but acts upon only one of the di-saccharides in this way, namely, lactose. Sucrose and maltose are not attacked by this organism, and Margaillan suggested that the latter should be used as a means of separating sucrose from lactose and dextrose. Testoni (*Ann. Lab. Centr. delle Gabelle*, 1912, page 581) has worked out a process of estimating sucrose in condensed milk and similar materials based on this principle. 20 gm. of condensed milk are dissolved in warm water and mixed with acetic acid to coagulate proteins, and then with basic lead acetate, the excess of lead being removed by means of a saturated solution of sodium sulphate. The solution is made up to 200 c.c., and an additional 2 c.c. of water are added to compensate for the volume of the coagulum. 100 c.c. of the filtrate are mixed with malt-peptone and 3 gm. of powdered marble, neutralised exactly, using phenolphthalein as indicator, and sterilised. The solution is inoculated with a pure culture of the bacillus and left for 6 days in an incubator at 35°. The lactic acid formed is precipitated by adding a slight excess of zinc sulphate and the volume made up to 100 c.c.; the solution is quickly filtered and the rotatory power measured with the polarimeter.

Jolles (*Zeitsch. Nahr. Genussm.*, 1910, **20**, 631) has stated that arabinose, rhamnose, dextrose, lævulose galactose, mannose, invert sugar, maltose and lactose are completely decomposed when heated with N/10 sodium hydroxide during 45 minutes in the water-bath or during 24 hours in a thermostat at 37°, acid substances being formed which are without action on polarised light; sucrose, on the other hand, is not in the least affected by this treatment, so that it is possible to estimate this substance in presence of the above-named sugars by treatment with sodium hydroxide

under the conditions named. Testoni states that Jolles' method gives the same results with sweetened wines, condensed milk and marmalades as are obtained by his process of fermentation (*cf.* Nowak, *Zeitsch. anal. Chem.*, 1912, 51, 610) and as the treatment with sodium hydroxide can be carried out rapidly, Jolles' method is preferable in most cases to the more tedious fermentation process. It is best to work at  $37^{\circ}$  rather than  $100^{\circ}$ , as at the lower temperature darkening of the solution and the consequent difficulty of reading the rotatory power of the solution are avoided. Before measuring the rotation the solution should be neutralised or made slightly acid with acetic acid, as otherwise the readings are slightly low. The nature of the change of rotation brought about by treatment with sodium hydroxide indicates whether commercial glucose or invert sugar was originally present. Bardach and Silberstein (*Zeitsch. Nahr. Genussm.*, 1911, 21, 540) have also applied Jolles' process to a number of commercial preparations.

## CANE SUGAR

Sucrose, saccharose.  $C_{12}H_{22}O_{11}$ .

Cane sugar is found in a very large number of plants, occurring both in the sap, seeds or fruits and in the milk of the cocoanut. Sucrose is manufactured from beet-root and the sugar cane and to a less extent from sorghum and the sugar maple.

It forms large transparent colourless crystals having the form of monoclinic prisms. These crystals have a sp. gr. of 1.55 to 1.61 according to the mode of crystallisation. Cane sugar has a rotation,  $[\alpha]_D = 66.5$ ; (see also page 369).

When cautiously heated it melts at about  $160^{\circ}$  and on cooling forms a transparent amber-coloured solid known as barley sugar. Heated at above  $160^{\circ}$  it decomposes.

Cane sugar dissolves in about half its weight of cold water, forming a very sweet viscid liquid known as syrup. (For information respecting the sp. gr. of sugar solutions see page 363.)

In boiling water it is soluble in all proportions. The boiling point of an aqueous sugar solution increases with the quantity of sugar dissolved as shown in the following table. The proportion of sugar present may thus be deduced from the boiling point.

## TABLE OF THE ELEVATION OF THE BOILING POINT OF SUGAR SOLUTIONS

(Claassen-Frentzel, *Deutsche Vereinzeitschrift*, 1893, p. 267.)

% sugar	Elevation of the b. p., F.	% sugar	Elevation of the b. p., F.
75.0	13.2	86.75	31.1
75.5	13.7	87.0	31.8
76.0	14.2	87.25	32.5
76.5	14.8	87.5	33.2
77.0	15.3	87.75	33.9
77.5	15.8	88.0	34.6
78.0	16.4	88.25	35.3
78.5	16.9	88.5	36.0
79.0	17.5	88.75	36.7
79.5	18.0	89.0	37.5
80.0	18.6	89.25	38.3
80.5	19.3	89.5	39.1
81.0	19.9	89.75	39.9
81.5	20.5	90.0	40.7
82.0	21.2	90.25	41.5
82.5	22.0	90.5	42.4
83.0	22.7	90.75	43.2
83.5	23.6	91.0	44.1
84.0	24.7	91.25	45.1
84.5	25.7	91.5	46.3
85.0	26.8	91.75	47.7
85.5	27.9	92.0	50.2
86.0	29.2		
86.25	29.8		
86.5	30.4		

When subjected to prolonged boiling the sugar acquires an acid reaction and becomes in part inverted.

Cane sugar is almost insoluble in absolute alcohol; in aqueous alcohol the solubility increases with the amount of water. The following table is due to Scheibler.

## SOLUBILITY OF SUGAR IN ALCOHOL OF DIFFERENT STRENGTHS

Per cent alcohol	100 c.c. of the solution contain	Sp. gr. of the saturated solution
0	85.8	1.3248
5	82.4	
10	79.4	1.2991
15	76.5	
20	73.4	1.236
25	69.8	
30	66.0	1.2293
35	61.6	
40	56.7	1.1823
45	51.6	
50	45.7	1.1294
55	39.6	
60	32.9	1.050
65	25.6	
70	17.8	0.9721
75	11.2	
80	6.4	0.8931
85	2.7	
90	0.7	0.8369
95	0.2	
97.4	0.08	
100	0.00	

**Sucrates.**—Cane sugar forms definite compounds with some metallic oxides. Thus lime, magnesia, and lead monoxide dissolve in syrup but are completely reprecipitated by passing a current of carbon dioxide through the liquid. Lead is attacked by sugar solutions, slowly in the cold, but more quickly at a boiling heat, the lead passing into solution. Several calcium sucates are known. The solution of calcium sucate has an alkaline and bitter taste, and forms the *liquor calcis saccharatus* of pharmacy. On mixing syrup with a concentrated solution of barium hydroxide, a crystalline precipitate is obtained, having the composition  $C_{12}H_{22}BaO_{12} = BaO, C_{12}H_{22}O_{11}$ , or  $C_{12}H_{21}(Ba.OH)O_{11}$ . This compound may be recrystallised from boiling water, separating in brilliant scales resembling boric acid. Its sparing solubility in cold water has been utilised in the treatment of saccharine juices, pure cane sugar being readily obtainable by decomposing the barium sucate by sulphuric acid. On adding strontium hydroxide to a boiling 15% solution of sugar, the compound  $C_{12}H_{20}(Sr.OH)_2O_{11}$  begins to separate, and when 2.5 molecules

of strontium hydroxide have been added almost the whole of the sugar will be precipitated. The granular sucrate may be washed with hot water, and decomposed with carbon dioxide. This process is employed in recovering sugar from molasses.<sup>1</sup>

Crystalline compounds are also easily obtained with some sodium salts; thus there are sodium chloride compounds,  $C_{12}H_{22}O_{11}$ ,  $NaCl$ ,  $2H_2O$  and  $2C_{12}H_{22}O_{11}$ ,  $3NaCl$ ,  $4H_2O$ , which have a lower optical rotation than corresponds to the sugar contained in them. The sodium iodide compound  $2C_{12}H_{22}O_{11}$ ,  $3NaI$ ,  $3H_2O$ , which may be obtained in large crystals, has an optical power directly proportional to that of the contained sugar.

(For further information with regard to the sucates, the reader is referred to Lippman's "*Chemie der Zuckerarten*.")

**Detection of Cane Sugar.**—Cane sugar is detected more readily by its physical properties than by its chemical reactions. The following are the leading characters of service in the recognition of cane sugar:

The sweet taste of the substance or the solution.

The dextrorotatory action of the solution.

The form of the crystals.

The characteristic odour produced on heating the solid substance.

The production of saccharic and oxalic acids by the action of moderately concentrated nitric acid.

The formation of alcohol by the prolonged action of yeast on the warm solution.

The increase in the reducing power of the liquid on Fehling's solution after inversion of the sugar by treatment with dilute acid, and the change in the rotatory power of the solution by inversion.

The similar change in the reducing and rotatory power of the solution by treatment with invertase. This reaction is very characteristic.

<sup>1</sup> For the extraction of *sucrose* from plant-products on a small scale, the fine substance should be boiled with strong alcohol, the solution filtered hot, and allowed to cool, when the cane sugar will usually crystallise out, or can be caused to do so after concentrating the solution. If *invert sugar* is also present, Peligot and Buignet recommend the following method: Add to the juice an equal volume of alcohol to prevent fermentation by keeping, filter, treat the filtrate with milk of lime in excess, and again filter. Boil the liquid, when calcium sucrate separates in amount corresponding to two-thirds of the whole cane sugar present. The precipitate is filtered off, well washed, diffused in water, and decomposed with carbon dioxide. The solution is filtered, evaporated at a gentle heat to a syrupy consistence, decolorised by animal charcoal, and mixed with strong alcohol till it becomes cloudy, when it is set aside to crystallise. If the solution, after treatment with carbon dioxide, yields a turbid filtrate, a solution of basic lead acetate is added, the liquid refiltered, and the excess of lead separated by hydrogen sulphide.

For information respecting the distinctive tests for *cane sugar*, *milk sugar*, *maltose*, and *hexoses* see pages 357 and 358.

## ANALYSIS AND VALUATION OF CANE AND BEET SUGAR PRODUCTS

**Refined Sugar.**—Refined white sugars are generally valued by their appearance, colour, etc., and they are generally of remarkably high purity, frequently polarising 99.9 or 99.95 and containing as little as 0.01 % of ash and 0.01 % of moisture. The complete analysis of refined sugars is rarely required.

Ultramarine is frequently added to refined sugars to correct any yellowish tint. It may be detected either by dissolving a considerable quantity, say 50 or 100 grm., of the sugar in water, filtering and examining the residue with a lens, or by spreading the sample on a sheet of white paper, examining it with a lens for crystals containing blue or dark specks and further examining such crystals under a higher magnifying power to confirm the colour.

**Raw Cane and Beet Sugar.**—High-grade raw cane sugar, intended for direct consumption, is, like white refined sugar, generally valued by its appearance. Raw cane or beet sugar bought for the purpose of refining, however, is bought sometimes on the basis of direct polarisation or more frequently on the “refining value” or yield (*rendement*) of refined sugar which is calculated from the results of full analysis. In the refining process, the impurities present in the raw sugar prevent crystallisation of part of the sugar, which remains in the form of molasses, and this effect of the impurities is known as the “melassigenic” effect. The melassigenic effect of the various constituents of raw sugars has been the subject of much investigation and controversy. The usual practice, however, is to ignore the melassigenic effect of the organic non-sugar and to consider only that due to the mineral matter and reducing sugars. In the case of raw cane sugars the yield is calculated by deducting the percentage of invert sugar plus 5 times the percentage of ash from the polarisation. For raw beet sugars the invert sugar percentage is multiplied by 3 if the percentage does not exceed 0.25 in the case of first product sugars or 0.5 in the case of second product sugars; if the percentage exceeds these amounts it is multiplied by 5; the percentage of ash is multiplied by 5 as in the case of cane sugars. Beet

sugars rarely contain invert sugar and if the amount of the latter is less than 0.05% it is recorded as "nil" or "trace," and no deduction therefor is made in calculating the yield. The following are fairly typical analyses of raw cane and beet sugars illustrating the calculation of the yield:

	RAW CANE SUGAR		RAW CANE SUGAR
Polarisation.....	98.10		93.80
Invert sugar.....	$0.71 \times 1 = 0.71$		$2.76 \times 1 = 2.76$
Ash.....	$0.30 \times 5 = 1.50$		$0.49 \times 5 = 2.45$
Moisture.....	0.49		1.69
Organic non-sugar.....	0.40	2.21	1.26
	<hr/>		<hr/>
Estimated yield.....	100.00		100.00
	95.89		88.59
	<hr/>		<hr/>
	RAW 1ST. PRODUCT BEET SUGAR		RAW 2ND. PRODUCT BEET SUGAR
Polarisation.....	95.40		91.40
Invert sugar.....	trace		$0.05 \times 3 = 0.15$
Ash.....	$0.85 \times 5 = 4.25$		$1.89 \times 5 = 9.45$
Moisture.....	2.36		3.27
Organic non-sugar.....	1.39		3.44
	<hr/>		<hr/>
Estimated yield.....	100.00		100.00
	91.15		81.80

It will be seen from the foregoing that the complete analysis of raw cane and beet sugar for refinery purposes involves determinations of the polarisation (direct), invert sugar and ash; the moisture is determined for purposes of control and the organic non-sugar is estimated by difference. The complete analysis is carried out as follows:

*Direct Polarisation.*—This is fully described on page 373. As there stated, the direct polarisation only corresponds with the percentage of sucrose when the sample is, like most raw beet sugars, practically free from invert sugar. In other cases, the method of double polarisation must be employed (see page 377) to determine the percentage of sucrose, but this is rarely required for commercial purposes.

*Invert Sugar.*—The percentage of invert sugar is generally determined by one of the volumetric processes described on page 392. Since raw beet sugars generally contain no invert sugar, a qualitative test should be made first. This is done by dissolving 2 or 3 grm. of the sample in water, adding 2 or 3 c.c. of Fehling's solution, and boiling the mixture for 1 or 2 minutes. If more than a trace of cuprous oxide is precipitated, a quantitative test must be made.

With a little experience the operator soon learns to distinguish between traces (or *nil*) and determinable quantities of invert sugar.

**Estimation of Ash.**—*The ash* of raw sugar may contain sand and other insoluble matters of mineral origin; various inorganic salts; and the non-volatile residues of the salts of various organic acids, among which may be acetic, succinic, oxalic, malic, tartaric, citric, aconitic (in cane sugar and juice only), aspartic (peculiar to beet sugar), saccharic, etc.

The most complete analysis of sugar-ash hitherto published is one by W. Wallace (*Chem. News*, 1878, 37, 76). The ash was derived from a Demerara cane sugar, the juice of which is supposed to have been treated with lime only. The raw sugar yielded 1.38% of ash, an analysis of which gave the following results:  $K_2O$ , 29.10;  $Na_2O$ , 1.94;  $CaO$ , 15.10;  $MgO$ , 3.76;  $Fe_2O_3$ , 0.56;  $Al_2O_3$ , 0.65;  $SiO_2$ , 12.38;  $P_2O_5$ , 5.59;  $SO_3$ , 23.75;  $CO_2$ , 4.06; and  $Cl$ , 4.15%. Total, 101.03; less  $O$  equal to  $Cl$ , 0.93 = 100.10.

The complete incineration of raw sugar is very difficult to effect satisfactorily, the ash obtained being very fusible, or light and easily blown away; and, as it consists largely of potassium carbonate, it is very deliquescent, and hence difficult to weigh accurately. To avoid these inconveniences, it is usual to treat the sugar with sulphuric acid before igniting it, by which means the ash obtained contains the bases as the comparatively little volatile, difficultly fusible, and non-deliquescent sulphates. An allowance is made for the increased weight of the ash due to the "sulphation" by deducting one-tenth of its weight.

The method of procedure is as follows: If not already wet or viscous, moisten from 2 to 4 grm. of the sample completely with the least possible quantity of water, and then with a little pure and concentrated sulphuric acid. Heat the whole gently till the frothing ceases and the mass forms a dry cinder. Ignite the charred mass in a muffle at a very low red heat, till the carbon is wholly consumed, and weigh when cold. If *sand* or *clay* be present in sensible quantity, it must be estimated by dissolving the ash in hydrochloric acid and weighing the insoluble residue. This must be deducted from the total ash before making the correction of  $\frac{1}{10}$ .

By this method, the bases being obtained as sulphates approximate more nearly in weight to that of the organic salts naturally present in the sugar which in the direct method are obtained as

carbonates. It has also been proposed to obtain the lead salts of the organic acids by precipitation with lead acetate, decompose these and titrate the acids set free with potassium hydroxide. The potassium combination approximates closely to the actual salts of the sugar.

The deduction of  $\frac{1}{10}$  from the weight of the sulphated ash to allow for "sulphation" has been shown by Schweizer (*Archief. Suikerind., Ned.-Indie*, 1916, 24, 214) and by Ogilvie and Lindfield (*Int. Sugar J.*, 1918, 20, 114) to be far from accurate. This method of calculation, however, is general.

The A. O. A. C. gives the following selection of official methods:

1. Heat from 5 to 10 gm. of the material (sugar, molasses, honey) in a platinum dish of from 50 to 100 c.c. capacity at 100° until the water is expelled, add a few drops of pure olive oil and heat slowly over a flame until intumescence ceases. The dish is then placed in a muffle and heated at low redness until a white ash is obtained. If the substance contains metal capable of uniting with platinum, a dish made of some other material must be used.

2. Carbonise the mass at a low heat, dissolve the soluble salts with hot water, burn the residual mass as above, add the solution of soluble salts, and evaporate to dryness at 100°; ignite gently, cool in a desiccator and weigh.

3. Saturate the sample with sulphuric acid, dry, ignite gently, then burn in a muffle at low redness. Deduct  $\frac{1}{10}$  of the weight of the ash, then calculate the percentage.

**Soluble and Insoluble Ash.**—Ignite the material according to method 1; add water to the ash in the platinum dish, heat nearly to boiling, filter through ash-free filter-paper, and wash with hot water until the filtrate and washings amount to about 60 c.c. Return the filter-paper and contents to the platinum dish, carefully ignite, and weigh. Compute percentages of water-insoluble ash and water-soluble ash.

**Alkalinity of Ash.**—The filtrate is titrated with N/10 hydrochloric acid and methyl orange. Excess of this acid is added to the insoluble ash in the platinum dish the contents of which are heated nearly to boiling, and, when cool, the excess is titrated with N/10 sodium hydroxide and methyl orange. The results are usually expressed as the amount of decinormal acid required by the ash of 1 gm. of sample.

The following figures illustrate the average composition of the ash of raw cane and beet sugars, according to Monier:

	Average composition of ash	
	Cane sugar	Beet sugar
Potassium (and sodium) carbonate.....	16.5	82.2
Calcium carbonate.....	49.0	6.7
Potassium (and sodium) sulphate.....	16.0	11.1
Sodium chloride.....	9.0	none
Silica and alumina.....	9.5	
	100.0	100.0

The following results by Scheibler are interesting, as showing the change produced in the weight and composition of sugar-ash by treatment with sulphuric acid:

	Beet-sugar ash	
	Original	Sulphated
Potassium oxide.....	25.65	25.65
Sodium oxide.....	21.62	21.62
Calcium oxide.....	6.53	6.53
Silica.....	0.72	0.72
Carbon dioxide.....	22.87	none
Sulphur trioxide.....	17.63	58.38
Chlorine.....	4.48	none
	99.50	112.90
Undetermined matters, and loss.....	0.50	less 1/10 11.29
	100.00	101.61

The following analyses by J. W. Macdonald (*Chem. News*, 1878, 37, 127) show the composition of the mixed sulphated ash obtained in the analysis of many samples of cane and beet sugar:

	Average sulphated ash	
	Cane sugar	Beet sugar
Potassium oxide.....	28.79	34.19
Sodium oxide.....	0.87	11.12
Calcium oxide.....	8.83	3.60
Magnesium oxide.....	2.73	0.16
Ferric oxide and alumina.....	6.90	0.28
Silica.....	8.29	1.78
Sulphur trioxide.....	43.65	48.85
	100.06	100.06

With respect to these analyses, it may be remarked that phosphates were not sought for by Macdonald, but representative samples showed 2.90% of this in the cane sugar ash, and only 0.24% in the ash of beet sugar. In the treatment of beet-juice it is usual to employ an excess of lime, which is afterwards removed by carbon dioxide. Hence, the phosphates of the juice would be precipitated almost entirely at an early stage of the manufacture. The proportion of phosphates in the ash of a sugar might perhaps furnish an indirect indication whether the article was manufactured from cane or from beet. Raw beet sugar, however, is readily distinguished from that derived from the cane by its appearance and flavor.

As any clay or sand contained in a sample of sugar has no prejudicial effect on the refining process, it is sometimes desirable to eliminate such extraneous matters before estimating the ash proper. This is done by dissolving a known weight of the sample in water, making the solution up to a known volume, filtering through a dry filter, evaporating one-half of the filtrate to dryness, moistening the residue with sulphuric acid, and igniting in the usual way.

**Estimation of Water in Commercial Sugars.**—*Water* is estimated in granular cane sugars by exposing 5 grm. of the sample in a thin layer to a temperature of  $60^{\circ}$ , and weighing every hour until there is no further loss. 12 hours are frequently required for complete desiccation. Beet sugars and good cane sugars may be dried at  $100^{\circ}$ , 2 hours being sufficient. Sugars containing much glucose generally give too high a result if dried at  $100^{\circ}$ , owing to a partial conversion of the glucose into glucosan and caramel. Large-grained refined sugars absorb moisture with great facility after drying, and should be weighed between closed watch-glasses.

Some operators prefer to employ a temperature of  $110^{\circ}$  for estimating the water in sugar, by which means the time required is usually greatly shortened.

The official A. O. A. C. method is as follows:

Dry from 2 to 5 grm. in a flat dish (nickel, platinum, or aluminium), at the temperature of boiling water, for 10 hours; cool in a desiccator and weigh; return to the oven and dry for an hour. If on weighing there be only a slight change of weight, the process may be considered finished; otherwise the drying must be continued until the loss of water in 1 hour is not great.

**Extractive Matters. Organic Matters Not Sugar.**—In ordinary commercial analyses of sugars, the sum of the sucrose, invert sugar, ash, and water is subtracted from 100.00, and the difference called "organic or undetermined matters." Under the last denomination are included many substances, of which the chief are: organic salts of the bases found in the ash; organic bases, such as asparagine and betaine; gummy and pectous bodies; proteins and enzymes; and insoluble organic matters, such as particles of cane. Some of these impurities have no interest for the sugar refiner, but others are very injurious. Thus the gummy matters interfere with the process of crystallisation, and the proteins tend to induce fermentation.

Although for most commercial purposes the estimation of these substances by difference is sufficient, the method is open to the objection that all the errors of the analysis are thrown on the organic matters, and that such a method makes no distinction between the harmless and injurious bodies comprised among the "organic matters not sugar." Hence even rough methods of obtaining a further knowledge of the nature and amount of these substances have an occasional value.

Walkoff obtains a comparative estimate of the organic matters in beet products by treating the solution of 5 grm. of the sugar in 200 c.c. of warm water with a solution of 2 grm. of pure tannin in 1000 c.c. The tannin solution is added from a burette, and samples of the liquid filtered from time to time, and the filtrate tested with ferrous sulphate, which gives a dark colour as soon as the tannin has been added in excess. The tannin is said to precipitate one-sixth of its weight of organic matters, but the process is chiefly valuable as a test for the comparative purity of different specimens. Asparagine is not estimated in this process. The sugar solution should be perfectly neutral.

Another comparative method consists in treating the solution of sugar with a slight excess of basic lead acetate, and weighing the precipitate produced, or the organic matters recoverable from it by decomposing it with sulphuretted hydrogen.

**Adulterations of Commercial Sucrose.**—Sugar may contain woody fibre from the crushed cane, much gritty sand, fungus spores and, when in bulk, all kinds of make-weights. The presence of sand and earthy matter is, of course, indicated by an excessive proportion of ash and the incomplete solubility in water.

Ultramarine is now frequently added to refined sugars to correct any yellowish tint. It may be easily detected by dissolving the sugar in cold water and allowing the suspended matter to settle.

**Fungus spores** are objectionable from the extreme rapidity with which, under suitable conditions, they develop into a spreading vegetable growth, especially in presence of nitrogenous matter. Such sugar is apt to undergo fermentation and turn sour, and preserves made with it soon spoil.

The *Acarus sacchari*, or sugar-mite, is a small animal closely resembling the itch-insect, and, like it, capable of burrowing under the skin

and producing an irritating pustular disease called the "grocer's itch," which attacks those employed in handling raw sugars.

**Starch-sugar** ("glucose") is employed as an adulterant of the lower grades of refined cane sugar. The starch-sugar used is commonly a highly converted kind, as the other varieties are too deliquescent to be suitable for the purpose. Anhydrous dextrose is sometimes employed, and the adulterated sugars generally contain less moisture than the genuine sugars of the same grades, which are known as "coffee sugars," and are always sold moist. The proportion of starch-sugar employed as an adulterant is usually about 20%.

If the sense of taste be first deadened by placing a pinch of pure powdered cane sugar on the tongue, and then, while the taste remains, a portion of the suspected sample tested in the same way, the bitterness of starch-sugar will be distinctly perceived if the specimen under examination be adulterated.

If the sample suspected to contain starch-sugar is placed in a beaker and stirred for a few seconds with rather less than its own weight of cold water, any hydrated dextrose will be seen floating in the liquid as white specks resembling crushed wheat. Anhydrous dextrose does not behave similarly, the crystals appearing as translucent as cane sugar.

When examined by means of Fehling's solution, genuine coffee sugar will rarely cause a reduction greater than corresponds to 5% of dextrose, whilst a sugar adulterated with the usual proportion of starch-sugar will show a reduction corresponding to about 20% of dextrose. Owing to the irregular composition of commercial starch-sugar, the proportion of it present in coffee sugar cannot be deduced with accuracy from the reducing power of the sample.

The same difficulty arises when an attempt is made to deduce the extent of adulteration from the optical activity of the sample; and, as commercial starch-sugar undergoes more or less change in its rotatory power by inversion with dilute acid, Clerget's method cannot be employed for the estimation of the sucrose present. Nevertheless, the polarimeter affords qualitative results of great value, and allows the fact of adulteration to be established beyond the possibility of doubt.

Some samples of coffee sugar adulterated with starch-sugar exert a rotation corresponding with upwards of 100% of cane sugar, owing

to the high rotatory power of maltose and dextrin. Such a result is sufficient to establish the presence of starch-sugar. In cases of adulteration with more highly converted starch-sugar, the direct polarimetric test will fail to indicate the existence of adulteration, but the fact will become manifest on inversion, which process will fail to produce the same change in the polarimetric reading that would ensue if only cane sugar and a small proportion of invert sugar were present.

Casamajor has proposed to utilise the fact that dextrose has a higher optical activity when freshly dissolved than after some time. The standard weight of sugar is dissolved in cold water, made up to 100 c.c. and the solution examined in the polarimeter with as little delay as possible. If the sugar is genuine, the rotation first observed will remain unchanged for any length of time, but if starch-sugar be present the rotatory power will gradually diminish. A sample examined by Casamajor showed 100.4 when first observed. In 15 minutes, the sugar-indication had fallen to 94.3; to 91.6 in 30 minutes; to 90.02 in 1 hour; to 89.7 in 3 hours; and to 89.3 in 5 hours, when it became stationary. After inversion, the sugar-indication was 72.7 (*Chem. News*, 1883, 48, 252).

**Molasses, Treacle and Golden Syrup.**—These by-products of the sugar industry should consist essentially of sucrose and invert sugar. They are often adulterated with glucose syrup. The production of molasses is due to the long-continued heating of the saccharine juice, but the quality varies with the nature and culture of the sugar-yielding plant, and with many other circumstances. "Refiners' molasses," the syrup obtained in the refining of sugar, retains a considerable amount of sucrose, the proportion being about 35% in cane-sugar molasses, and as much as 50% in that from beet-root. This is prevented from crystallising by the impurities present in the raw sugar. The molasses from raw cane sugar contains a considerable percentage of invert sugar, from which beet-root molasses is comparatively free, but the latter contains raffinose, aspartic acid, and some other substances. The proportion of salts contained in beet-root molasses is usually 10 to 14%, whereas refiners' treacle from raw cane sugar rarely contains half that proportion.

The following analyses show the general composition of molasses:

	Su- crose	Invert sugar	Ash	Water	Organic matters other than sugar	Authority
<b>Sugar-cane Products:</b>						
Green syrup.....	62.7	8.0	1.0	27.7	0.6	W. Wallace.
Golden syrup.....	39.6	33.0	2.5	22.7	2.8	W. Wallace.
Treacle.....	32.5	37.2	3.5	23.4	3.5	W. Wallace.
Molasses.....	48.0	18.0	1.4	31.1	18.0	W. Wallace.
Molasses, average.....	35.0	10.0	5.0	20.0	10.0	J. H. Tucker.
Molasses, refiners'.....	37.5	.....	.....	25.0	.....	Casamajor.
<b>Beet-root Products:</b>						
Molasses.....	50.9	1.1	12.9	19.0	16.1	Houghton and Gill.
Molasses, average.....	50.0	.....	10.0	20.0	20.0	Wigner and Harland.
Molasses, average.....	55.0	trace	12.0	20.0	13.0	J. H. Tucker.
Molasses, average.....	49.4	.....	.....	17.1	.....	Payen.

Bodenbender found an average of 1.5% of nitrogen in beet-root molasses, of which nearly 1% existed as betaine and proteins, and nearly the whole of the remainder as aspartic and glutamic acids and asparagine.

Vanillin has been recently recognised in beet-sugar molasses and may even be extracted from many samples of raw sugar by simple agitation with ether.

The analysis of cane and beet syrups and molasses generally involves estimations of sucrose, invert sugar, ash and water, whilst in the case of beet molasses the estimation of raffinose is sometimes required.

*Sucrose*.—Since syrups and molasses generally contain a considerable proportion of reducing sugar, the estimation of sucrose cannot be made by direct polarisation and recourse must be made to the Clerget method or one of the modifications of it; these are fully described on page 377.

*Invert sugar* is estimated by one of the methods described on page 392.

*Ash* is estimated by one of the methods described on pages 439, 440.

*Water*.—The estimation of water in treacle, beet, cane juice, and similar articles is tedious, owing to the low temperature which must be employed, and to the formation of a skin on the surface of the liquid. To avoid this 5 grm. (or other known weight) of the sample should be dissolved in water, and the solution made up to 100 c.c. 10 c.c. of this solution (= 0.5 grm. of the original sample) are poured over about 12 or 15 grm. of previously ignited silver-sand, contained in a flat dish. The whole is dried at a temperature not exceeding

60° until constant, the increase in weight being due to the dry sugar in 0.5 grm. of the sample. By conducting the desiccation in a partial vacuum, from which the moisture is removed by sulphuric acid or calcium chloride, the operation may be finished in a few hours.

The official A. O. A. C. methods are as follows:

**1. In Masecutes, Molasses, Honeys, and Other Liquid and Semi-liquid Products.**—Prepare pumice stone in two grades of fineness. One of these should pass through a 1 mm. sieve, whilst the other should be composed of particles too large for a 1 mm. sieve, but sufficiently small to pass through a sieve having meshes 6 mm. in diameter. Make the determination in flat metallic dishes or in shallow, flat-bottomed weighing bottles. Place a layer of the fine pumice stone 3 mm. in thickness over the bottom of the dish, and upon this place a layer of the coarse pumice stone from 6 to 10 mm. in thickness. Dry the dish thus prepared and weigh. Dilute the sample with a weighed portion of water in such a manner that the diluted material shall contain from 20 to 30% of dry matter. Weigh into the dish, prepared as described above, such a quantity of the diluted sample as will yield, approximately, 1 grm. of dry matter. Use a weighing bottle provided with a cork through which a pipette passes if this weighing cannot be made with extreme rapidity. Place the dish in a water-oven and dry to constant weight at the temperature of boiling water, making trial weighings at intervals of 2 hours. In case of materials containing much lævulose or other readily decomposable substances, conduct the drying *in vacuo* at a lower temperature. In the case of very unstable material, the temperature can safely be lowered to 70°.

**2. Method for Drying Molasses with Quartz Sand.**—In a flat-bottomed dish place 6 or 7 grm. of pure quartz sand and a short stirring rod. Dry thoroughly, cool in a desiccator, and weigh. Then add 3 or 4 grm. of the molasses, mix with the sand, and dry at the temperature of boiling water for from 8 to 10 hours. Stir at intervals of an hour; then cool in a desiccator and weigh. Stir, heat again in the water-oven for an hour, cool and weigh. Repeat heating and weighing until the loss of weight in 1 hour is not greater than 3 mg. The sand used should be pure quartz. It should be digested with strong hydrochloric acid, washed, dried, and ignited, and kept in a stoppered bottle.

The amount of water in molasses, honey, and similar materials may also be calculated from the sp. gr. or solution density (see page 363), but a correction must be made for the influence of the mineral matter present, since solutions of most salts have a much higher sp. gr. than solutions of sugars of the same concentration. Thus, the average solution density for the mineral matter in sugar products is 8, whereas that for a mixture of sucrose and invert sugar may be taken as 3.86. Hence, to determine the water in molasses, etc., the following procedure is employed:

10 grm. of the sample are dissolved and made up to 100 c.c., and the sp. gr. of this solution at 15.5°/15.5° is determined, water being taken as 1000. Then

$$\text{Water} = 100 - \left\{ \frac{(\text{sp. gr.} - 1000) - (\% \text{ ash} \times 0.8)}{3.86} + \% \text{ ash} \right\}$$

The refractometric method for determining water in liquid sugar products is both accurate and expeditious (see page 390.)

**Raffinose.**—This sugar which occurs in beet molasses is estimated by the method described on page 383.

**Glucose.**—This is the non-fermentable reducing sugar or mixture of carbohydrates formed from hexoses by the action of alkalis. Beet molasses contains little or no glucose, but cane molasses sometimes contains as much as 5%. H. Pellet (*Ann. Chim. anal.*, 1917, 22, 43) proposes the following method for its estimation:

100 grm. of the molasses are diluted to about 1000 c.c., slightly acidified, and fermented for 3 days with 50 grm. of bottom fermentation yeast. The fermented liquid is defecated with normal lead acetate, freed from excess of lead with sodium carbonate, and its reducing power determined by heating with Fehling's solution at 63° to 65° for 30 minutes. The reducing power of glucose is assumed to be half that of invert sugar.

**Sugar Confectionery.**—Analysis of sweets is generally a question of the detection of poisonous colouring materials. The percentage of sugar present may be estimated in the usual way and the presence of starch-sugar ascertained as described (see page 444). Starch-sugar is very extensively employed in the manufacture of confectionery.

Essences may be dissolved out by means of petroleum spirit (gasoline) and identified by their odour; those now used are often artificial.

Treatment of the colouring matter with alcohol, with water, and with bleaching powder quickly characterises it as organic or inorganic in nature.

Among the red colouring matters of sugar confectionery, red lead and vermilion have been observed, but in most cases harmless organic pigments are employed.

Lead chromate has been employed as a yellow colouring agent. Greens have been found to be produced by a mixture of lead chromate and Prussian blue, and copper arsenite, and other copper pigments have also been met with. The blue mineral colouring matters may be Prussian blue or ultramarine. The detection of the injurious colouring matters in confectionery belongs to mineral analysis, and requires no detailed description here.

Candies and confectons are now almost invariably coloured with coal-tar products, prepared especially for the purpose and free from metallic impurities. In most cases very small amounts of colour are used.

Under a regulation issued in accordance with the provisions of the U. S. (Federal) food law the following seven colours are permitted in candies and confections, the manufacture and sale of which are within the jurisdiction of the law. The numbers refer to Schultz & Julius' *Systematic Survey of Organic Colouring Matters* (translated by Green):

- 107 Carmosine B.
- 56 Scarlet 40.
- 517 Eosin B. C.
- 85 Orange G.
- 4 Yellow F. Y.
- 435 Acid green GG.
- 692 Indigotin.

The manufacturer of the said dyes is required to guarantee that they really are what they are represented to be, that they are not mixtures and that they do not contain harmful impurities.

### Sugar-cane and Beet Juices

The juice obtained by crushing and pressing the sugar-cane<sup>1</sup> has usually a sp. gr. of 1.070 to 1.090, but has been met with as low as 1.046 and as high as 1.110. It is an opaque, frothy, yellowish-green liquid. On filtration it yields a pale yellow fluid, which is nearly pure syrup, the greenish scum containing chlorophyll, a peculiar wax called cerosin, protein matters, fibre, and a considerable proportion of mineral matter. The pure or nearly colourless juice from which the green matter has been separated contains, on the average, in 100 parts: water, 81.00; sugar, 18.20; organic matters precipitated by lead salts, 0.45; and mineral matters, 0.35.

<sup>1</sup> The following analyses show the general percentage composition of the sugar-cane.

Locality and kind of cane	Water	Sugar	Woody fibre	Salts	Authority
Martinique.....	72.1	18.0	9.9		Pelilot.
Guadeloupe.....	72.0	17.8	9.8	0.4	Dupuy.
Havana.....	77.0	12.0	11.0		Casaseca.
Cuba.....	65.9	17.7	16.4		Casaseca.
Mauritius.....	69.0	20.0	10.0	1.0	Icery.
Ribbon cane.....	76.73	13.39	9.07	0.39	Avequin.
Tahiti.....	76.08	14.28	8.87	0.35	Avequin.

The following is a more detailed analysis, by Payen, of Otaheite cane at maturity:

Water.....	71.04
Sugar.....	18.00
Cellulose, ligneous matter, pectin, and pectic acid.....	9.56
Proteins.....	0.55
Cerosin; red, green, and yellow colouring matters; fatty matter; resins; essential oil; aromatic matter; and a deliquescent substance.....	0.37
Insoluble salts, 0.12; soluble, 0.16, consisting of phosphates, sulphates, chlorides, oxalates, acetates, malates.....	0.28
	99.80

According to Casaseca, the lower portions of the sugar-cane are the richest in sugar, the centre being of about the average composition. This is shown by the following analysis by Gill of carefully sampled good average cane from the Aska district, Madras:

	A	B	C
	Two feet top, %	Two feet middle, %	Two feet root, %
Megass proper.....	7.63	8.47	8.30
Juice.....	92.37	91.58	91.70
Containing, cane sugar.....	10.63	13.31	13.37
Containing, invert sugar.....	2.64	1.51	1.54

The sp. gr. of the juice from the white beet<sup>1</sup> is usually between 1060 and 1070, occasionally reaching 1078. Beet juice contains a large amount of foreign matters in proportion to the sugar, a fact that renders the manufacture of sugar from beet-root much more troublesome than from cane. The average percentage composition of expressed beet juice is approximately: water, 82.68; sugar, 11.25; other organic matters, 1.47; and mineral matters, 0.67.

The analysis of cane and beet juices may be effected by the methods described under "Molasses, Treacle and Golden Syrup."

## SUCROSE IN BEETROOT

**Estimation of Sucrose in the Beet.**—The process of sampling the roots which may amount to a considerable number involves the use of a special rasping machine, *e.g.*, that constructed by Kiehle of Leipzig by means of which a longitudinal section of each root is reduced to pulp. The portions of pulp from each root are well mixed and used for analysis by one of the following methods:

The expressed juice had the following composition:

	A	B	C
Cane sugar.....	11.51	14.55	14.58
Invert sugar.....	2.86	2.65	1.68
Ash.....	0.33	0.28	0.25
Unknown.....	0.50	0.92	0.49
Apparent solids.....	15.20	17.40	17.00
Water.....	84.80	82.60	83.00
	100.00	100.00	100.00

The megass referred to above contains little but woody fibre, as the sugar is extracted in the Aska district by the diffusion process. Ordinary megass or milltrash after passing the rollers retains 8 or 10% of sugar and 50% of water.

The ash of the sugar-cane contains about 50% of silica, 5 to 8% of phosphoric acid, and different proportions of potassium. Sodium appears to be a constant constituent.

<sup>1</sup> The following is an analysis by Payen of the white or sugar beet:

Water.....	82.7
Sugar.....	11.3
Cellulose.....	0.8
Proteins.....	1.5
Fatty matter.....	0.1
Pectin matters, asparagine, aspartic acid, betaine; oxalates, nitrates, phosphates.....	3.7

100.1

**Alcoholic Extraction.**<sup>1</sup>—26 grm. of the finely divided pulp are treated with 3 c.c. of basic lead acetate solution and extracted with 90% alcohol in a Soxhlet extractor until free from sucrose as shown by the resorcinol and sulphuric acid test. The extract is then diluted to 100 c.c. at 20° with alcohol and polarised in the usual way. This method leaves nothing to be desired on the score of accuracy but it is too elaborate and tedious for most commercial purposes.

**Cold Aqueous Digestion.**—26 grm. of the beet pulp, which must be very finely divided, are treated with 5 or 6 c.c. of basic lead acetate solution, made nearly to volume, well mixed by rotating the flask, allowed to stand for 25 minutes to insure expulsion of air bubbles, made up to 200.6 c.c. (the 0.6 c.c. compensating for the volume of the marc or fibre), filtered and polarised in a 200 or 400 mm. tube. This method is rapid but, owing to the difficulty of removing air-bubbles, it is less accurate than that of hot digestion.

**Herzfeld's Modification of the Sachs-le-Docte Method of Hot Aqueous Digestion.**—26 grm. of pulp are transferred to a nickel-plated iron vessel which is of cylindrical shape with narrowed neck; 177 c.c. of dilute basic lead acetate solution (5 parts of basic lead acetate solution of 53.5° Brix to 100 parts of water) are added, the mixture is shaken and the vessel, loosely stoppered with a tinfoil-covered stopper, is placed in a water-bath at 75° to 80° for 30 minutes. The vessel is shaken intermittently during heating and when all the air has been expelled (generally in 5 minutes) the stopper is tightened. After heating, the vessel is removed from the bath, cooled and shaken thoroughly. The stopper is then removed, the solution filtered and polarised in a 400 mm. tube after addition of a drop of acetic acid. According to this procedure, it is assumed that 26 grm. of pulp contain  $(200-177) = 23$  c.c. of juice and the reading in a 400 mm. tube gives the sucrose-content directly. The method gives very accurate results (*cf.* Levitcki, *Zentr. Zuckerind.*, 1911, 20, 357).

Methods of estimating sucrose in beet pulp by digestion with cold or hot alcohol have also been used but they are less satisfactory than those described above.

<sup>1</sup>*Cf.* A. Herzfeld, *Zeitsch. Ver. deut. Zuckerind.*, 1901, 51, [2], 334.

## MALTOSE

Maltose is the chief product of degradation of starch and also occurs in the leaves of some plants. It usually occurs in fine crystalline needles of the hydrate  $C_{12}H_{22}O_{11} \cdot H_2O$ . The amorphous anhydride is very hygroscopic. A convenient method for the preparation pure maltose is given by Baker and Day (*Analyst*, 1908, **33**, 393). Maltose is hydrolysed to two molecules of dextrose when heated with dilute acids, but is far more stable than sucrose (see page 358). Hydrolysis takes place more rapidly under the influence of a specific enzyme, *maltase*.

Maltose may be distinguished from dextrose by its neutral behaviour towards copper-acetate solution (Barfoed's reagent, see page 426) and by the solubility of its osazone in hot water.

On heating with phenylhydrazine at the temperature of the water-bath, dextrose or lævulose gives a precipitate of the phenylosazone after 10 minutes, but maltose forms a precipitate only on cooling the solution after an hour's heating. The osazones of the two sugars can thus be separated easily from a solution containing them both. Maltosazone is soluble in about 75 parts of hot water, whereas glucosazone is almost insoluble. When testing a freshly prepared mixture of osazones in this manner, it is essential to wash them first thoroughly with water and benzene so as to remove products which tend to make glucosazone appear soluble. Maltosazone is soluble in a cold mixture of equal parts of water and acetone.

It has been frequently proposed to estimate maltose by hydrolysis with dilute hydrochloric or sulphuric acid at  $100^\circ$ , noting the change of cupric reducing power or specific rotatory power of the solution after allowing for the inversion of cane sugar present. Under carefully regulated conditions this method gives approximate results in the case of pure maltose or a mixture of maltose and dextrose (*cf.* Baker and Dick, *Analyst*, 1905, **30**, 79) but, as shown by Davis and Daish (*J. Agric. Sci.*, 1913, **5**, 453) it is quite useless when sucrose or lævulose is also present, owing to the large amount of decomposition of the latter sugar which is caused by the prolonged heating with dilute acids. In the paper cited, it is shown that it is impossible to effect complete hydrolysis of maltose by dilute acid at  $100^\circ$ , without at the same time destroying a large proportion of the

lævulose which is present; thus 31% of the lævulose present in a 1% solution is destroyed when this solution is heated with 2.4% hydrochloric acid during 2 hours, this being the time necessary to effect 98% of complete hydrolysis of maltose in a 1% solution of this sugar. Even at 70° it is impossible completely to hydrolyse maltose in presence of sucrose or lævulose without at the same time destroying considerable quantities of lævulose; thus in 1% solution of the sugar, even after 24 hours' heating with a 2.4% solution of hydrochloric acid, only 94% of the maltose is hydrolysed, whilst more than 5% of the lævulose is destroyed.

It is therefore impossible to estimate maltose with any degree of accuracy by acid hydrolysis when sucrose or lævulose is also present. In such cases it is necessary to employ a biochemical method, involving the use of maltase-free yeasts. Davis and Daish (*loc. cit.*) have suggested the following process, which has been thoroughly tested and found to give quite accurate results; it was shown that it is possible to ferment away every trace of dextrose, lævulose, or sucrose by means of any one of the three special yeasts *Saccharomyces marxianus*, *S. exiguus*, or *S. anomalus* and to recover every trace of maltose, which can then be estimated by measuring the residual reducing power.

To 50 c.c. of the solution containing the sugars (previously purified by means of basic lead acetate and then freed from lead as described below), which must be very faintly acid to litmus paper, 5 c.c. of yeast water are added; the solution is then sterilised in a flask closed with a cotton-wool stopper, by heating during 20 minutes in the autoclave at 115°–120°, and is then inoculated with a trace of the pure maltase-free yeast and incubated at 25° for 3 to 4 weeks. When the fermentation is complete<sup>1</sup> 5 c.c. of alumina cream are added and the solution well boiled; it is then filtered and the precipitate well washed until the filtrate has a volume of 100 c.c. An aliquot portion (50 c.c.) can then be used for measuring the reducing power.

For the yeasts to grow satisfactorily it is necessary to make sure that every trace of lead has been removed from the solution. The best method of effecting this is to precipitate the excess of lead by adding sodium carbonate, little by little, until no further precipitate is produced, then to make the filtrate slightly acid with hydrochloric acid and to precipitate the traces of lead remaining in solution by

<sup>1</sup> 0.20 to 0.5 grm. of sucrose are invariably completely fermented under the above conditions after 3 weeks.

hydrogen sulphide. The lead can also be removed directly from the solution by using hydrogen sulphide without the prior employment of sodium carbonate, but in such cases the solution usually becomes strongly acid if much lead acetate be present (owing to the liberation of acetic acid) and this acidity must be very nearly neutralised by adding sodium carbonate before sterilising and inoculating with the yeast. In all cases, hydrogen sulphide is expelled by sucking air through the solution obtained after filtering from the lead sulphide; the solution which is used for the fermentations should show a faint but distinct acid indication to litmus.

When small quantities of pentoses are present in the solutions to be analysed (as is frequently the case in dealing with plant extracts) it is necessary to introduce a correction for these; the pentoses are very slowly, if at all, fermented by the maltase-free yeasts and consequently, like maltose itself, exercise a reducing effect on the Fehling's solution. The correction for the pentoses is obtained by carrying out fermentations with a pure culture of ordinary distillery or brewers' yeast which ferments away the maltose but leaves the pentoses; the slight residual reducing power found after carrying out a fermentation with such yeast is indeed a measure of the pentoses present and can be used as a means of verifying the presence of these substances or of estimating them in cases when the ordinary pentose method is likely to give incorrect results owing to the presence of other sugars (Davis and Sawyer, *J. Agric. Sci.*, 1914, 6, 406). On subtracting the value found for the reducing power remaining after fermentation with ordinary yeast from the value given by the maltase-free yeasts, the cupric reduction due to the maltose alone is obtained.

*S. anomalus* does not lend itself quite so well to quantitative experiments as the other maltase-free yeasts, as it is slower in its action and is less efficient as a sugar-remover—that is, a greater growth of yeast is necessary for the removal of a certain weight of sugar. Moreover, the Fehling's solution in the subsequent reduction often filters very slowly and the cuprous oxide is also generally somewhat contaminated by traces of copper compounds formed by the action of the Fehling's solution on substances elaborated during the growth of the yeast. It is therefore best to make use of *S. exiguus* and *S. marxianus* only. On page 564 is given a general scheme for the analysis of plant material, showing the way in which this method is applied in such cases.

## LACTOSE

Milk sugar or lactose reduces Fehling's copper solution, the reducing power being roughly three-quarters that of dextrose. It rapidly reduces ammoniacal silver nitrate. The osazone is soluble in boiling water and it may thus be detected in presence of the glucoses or galactose. Characteristic of lactose and galactose is the formation of mucic acid when oxidised by nitric acid. Use is often made of the low solubility in water and facility of crystallisation to identify lactose. According to E. Saillard (*Chim. et Ind.*, 1919, 2, 1035) 1 litre of water dissolves 200 grm. of lactose at  $21.5^{\circ}$ , and 770 grm. at  $65^{\circ}$ , and the same authority states that lactose may be completely hydrolysed by heating a solution of 5 grm. of the sugar in 100 c.c. of water with 10 c.c. of hydrochloric acid (sp. gr. 1.19) at  $90^{\circ}$  for 90 minutes.

In practice the estimation of lactose is required simply in milk and products such as condensed milk, whey, koumiss and kefir derived therefrom.

A method which affords an approximate estimation of the sugar in milk consists in adding a few drops of acetic acid and warming, filtering from the resultant curd, boiling, evaporating the clear whey to a small bulk, again filtering, and then evaporating the filtrate to dryness. The residue, after drying at  $130^{\circ}$ , consists almost wholly of lactose and salts. The amount of the former substance present may be ascertained by igniting the weighed residue and noting the loss of weight. The amount of sugar thus obtained is always a little too high.

**Estimation of Milk Sugar by Gravimetric Methods.**—In estimating lactose in milk by Fehling's solution it is necessary to remove the proteins. This may be done by warming with a few drops of acetic acid, filtering, boiling the filtrate to coagulate the remaining proteins and again filtering. This filtrate is neutralised before adding the copper solution. It is better, however, to precipitate the proteins with copper sulphate.

Soxhlet's method adopted by the A. O. A. C. is as follows:

**1. Preparation of the Milk Solution.**—Dilute 25 c.c. of the milk with 400 c.c. of water and add 10 c.c. of a solution of copper sulphate of the strength given for Soxhlet's modification of Fehling's solution, page 392; add about 7.5 c.c. of a solution of potassium hydroxide of

such strength that 1 volume of it is just sufficient to precipitate completely the copper as hydroxide from 1 volume of the solution of copper sulphate. Instead of a solution of potassium hydroxide of this strength 8.8. c.c. of a half-normal solution of sodium hydroxide may be used. After the addition of the alkali solution the mixture must still have an acid reaction and contain copper in solution. Fill the flask to the 500 c.c. mark, mix, and filter through a dry filter.

**2. Estimation.**—This is carried out by the method of Munson and Walker described on p. 401 and the following table, compiled by the same chemists, is used (*cf.* P. H. Walker, *J. Amer. Chem. Soc.*, 1912, 34, 202).

TABLE FOR CALCULATING LACTOSE  
(Expressed in milligrams)

Cu- prous oxide (Cu <sub>2</sub> O)	Cop- per (Cu)	Lactose			Cu- prous oxide (Cu <sub>2</sub> O)	Cop- per (Cu)	Lactose		
		(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> ½ H <sub>2</sub> O)	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> , H <sub>2</sub> O)			(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> ½ H <sub>2</sub> O)	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> , H <sub>2</sub> O)
10	8 9	3 8	3 9	4 0	45	40 0	26 8	27 5	28 2
11	9 8	4 5	4 6	4 7	46	40 9	27 4	28 2	28 9
12	10 7	5 1	5 3	5 4	47	41 7	28 1	28 9	29 6
13	11 5	5 8	5 9	6 1	48	42 6	28 7	29 5	30 3
14	12 4	6 4	6 6	6 8	49	43 5	29 4	30 2	31 0
15	13 3	7 1	7 3	7 5	50	44 4	30 1	30 9	31 7
16	14 2	7 8	8 0	8 2	51	45 3	30 7	31 5	32 4
17	15 1	8 4	8 6	8 9	52	46 2	31 4	32 2	33 0
18	16 0	9 1	9 3	9 5	53	47 1	32 1	32 9	33 7
19	16 9	9 7	10 0	10 2	54	48 0	32 7	33 6	34 4
20	17 8	10 4	10 7	10 9	55	48 9	33 4	34 3	35 1
21	18 7	11 0	11 3	11 6	56	49 7	34 0	34 9	35 8
22	19 5	11 7	12 0	12 3	57	50 6	34 7	35 6	36 5
23	20 4	12 3	12 7	13 0	58	51 5	35 4	36 3	37 2
24	21 3	13 0	13 4	13 7	59	52 4	36 0	37 0	37 9
25	22 2	13 7	14 0	14 4	60	53 3	36 7	37 6	38 6
26	23 1	14 3	14 7	15 1	61	54 2	37 3	38 3	39 3
27	24 0	15 0	15 4	15 8	62	55 1	38 0	39 0	40 0
28	24 9	15 6	16 1	16 5	63	56 0	38 6	39 7	40 7
29	25 8	16 3	16 7	17 1	64	56 8	39 3	40 3	41 4
30	26 6	16 9	17 4	17 8	65	57 7	40 0	41 0	42 1
31	27 5	17 6	18 1	18 5	66	58 0	40 6	41 7	42 8
32	28 4	18 3	18 7	19 2	67	59 5	41 3	42 4	43 5
33	29 3	18 9	19 4	19 9	68	60 4	41 9	43 1	44 2
34	30 2	19 6	20 1	20 6	69	61 3	42 6	43 7	44 8
35	31 1	20 2	20 8	21 3	70	62 2	43 3	44 4	45 5
36	32 0	20 9	21 4	22 0	71	63 1	43 9	45 1	46 2
37	32 9	21 5	22 1	22 7	72	64 0	44 6	45 8	46 9
38	33 8	22 2	22 8	23 4	73	64 8	45 2	46 4	47 6
39	34 6	22 8	23 5	24 1	74	65 7	45 9	47 1	48 3
40	35 5	23 5	24 1	24 8	75	66 6	46 6	47 8	49 0
41	36 4	24 2	24 8	25 4	76	67 5	47 2	48 5	49 7
42	37 3	24 8	25 5	26 1	77	68 4	47 9	49 1	50 4
43	38 2	25 5	26 2	26 8	78	69 3	48 5	49 8	51 1
44	39 1	26 1	26 8	27 5	79	70 2	49 2	50 5	51 8

TABLE FOR CALCULATING LACTOSE—*Continued*

Cu- prous oxide (Cu <sub>2</sub> O)	Cop- per (Cu)	Lactose			Cu- prous oxide (Cu <sub>2</sub> O)	Cop- per (Cu)	Lactose		
		(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> ½H <sub>2</sub> O)	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> , H <sub>2</sub> O)			(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> ¾H <sub>2</sub> O)	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> , H <sub>2</sub> O)
80	71.1	49.9	51.2	52.5	135	119.9	86.2	88.5	90.8
81	71.9	50.5	51.9	53.2	136	120.8	86.9	89.1	91.5
82	72.8	51.2	52.5	53.9	137	121.7	87.5	89.8	92.1
83	73.7	51.8	53.2	54.6	138	122.6	88.2	90.5	92.8
84	74.6	52.5	53.9	55.3	139	123.5	88.9	91.2	93.5
85	75.5	53.1	54.6	56.0	140	124.4	89.5	91.9	94.2
86	76.4	53.8	55.2	56.6	141	125.2	90.2	92.5	94.9
87	77.3	54.5	55.9	57.3	142	126.1	90.8	93.2	95.6
88	78.2	55.1	56.6	58.0	143	127.0	91.5	93.9	96.3
89	79.1	55.8	57.3	58.7	144	127.9	92.2	94.6	97.0
90	79.9	56.4	58.0	59.4	145	128.8	92.8	95.3	97.7
91	80.8	57.1	58.6	60.1	146	129.7	93.5	95.9	98.4
92	81.7	57.8	59.3	60.8	147	130.6	94.2	96.6	99.1
93	82.6	58.4	60.0	61.5	148	131.5	94.8	97.3	99.8
94	83.5	59.1	60.7	62.2	149	132.3	95.5	98.0	100.5
95	84.4	59.7	61.3	62.9	150	133.2	96.1	98.7	101.2
96	85.3	60.4	62.0	63.6	151	134.1	96.8	99.3	101.9
97	86.2	61.1	62.7	64.3	152	135.0	97.5	100.0	102.6
98	87.0	61.7	63.4	65.0	153	135.9	98.1	100.7	103.3
99	87.9	62.4	64.0	65.7	154	136.8	98.8	101.4	104.0
100	88.8	63.0	64.7	66.4	155	137.7	99.5	102.1	104.7
101	89.7	63.7	65.4	67.1	156	138.6	100.1	102.8	105.4
102	90.6	64.4	66.1	67.8	157	139.5	100.8	103.4	106.1
103	91.5	65.0	66.7	68.5	158	140.3	101.5	104.1	106.8
104	92.4	65.7	67.4	69.1	159	141.2	102.1	104.8	107.5
105	93.3	66.4	68.1	69.8	160	142.1	102.8	105.5	108.2
106	94.2	67.0	68.8	70.5	161	143.0	103.4	106.2	108.9
107	95.0	67.7	69.5	71.2	162	143.9	104.1	106.8	109.6
108	95.9	68.3	70.1	71.9	163	144.8	104.8	107.5	110.3
109	96.8	69.0	70.8	72.6	164	145.7	105.4	108.2	111.0
110	97.7	69.7	71.5	73.3	165	146.6	106.1	108.9	111.7
111	98.6	70.3	72.2	74.0	166	147.4	106.8	109.6	112.4
112	99.5	71.0	72.8	74.7	167	148.3	107.4	110.3	113.1
113	100.4	71.6	73.5	75.4	168	149.2	108.1	110.9	113.8
114	101.3	72.3	74.2	76.1	169	150.1	108.8	111.6	114.5
115	102.1	73.0	74.9	76.8	170	151.0	109.4	112.3	115.2
116	103.0	73.6	75.6	77.5	171	151.9	110.1	113.0	115.9
117	103.9	74.3	76.2	78.2	172	152.8	110.8	113.7	116.6
118	104.8	75.0	76.9	78.9	173	153.7	111.4	114.3	117.3
119	105.7	75.6	77.6	79.6	174	154.6	112.1	115.0	118.0
120	106.6	76.3	78.3	80.3	175	155.4	112.8	115.7	118.7
121	107.5	76.9	79.0	81.0	176	156.3	113.4	116.4	119.4
122	108.4	77.6	79.6	81.7	177	157.2	114.1	117.1	120.1
123	109.3	78.3	80.3	82.4	178	158.1	114.8	117.8	120.8
124	110.1	78.9	81.0	83.1	179	159.0	115.4	118.4	121.5
125	111.0	79.6	81.7	83.8	180	159.9	116.1	119.1	122.2
126	111.9	80.3	82.4	84.5	181	160.8	116.7	119.8	122.9
127	112.8	80.9	83.0	85.2	182	161.7	117.4	120.5	123.6
128	113.7	81.6	83.7	85.9	183	162.5	118.1	121.2	124.3
129	114.6	82.2	84.4	86.6	184	163.4	118.7	121.8	125.0
130	115.5	82.9	85.1	87.3	185	164.3	119.4	122.5	125.7
131	116.4	83.6	85.7	88.0	186	165.2	120.1	123.2	126.4
132	117.2	84.2	86.4	88.7	187	166.1	120.7	123.9	127.1
133	118.1	84.9	87.1	89.4	188	167.0	121.4	124.6	127.8
134	119.0	85.5	87.8	90.1	189	167.9	122.1	125.3	128.5

TABLE FOR CALCULATING LACTOSE—Continued

Cu- prous oxide (Cu <sub>2</sub> O)	Cop- per (Cu)	Lactose			Cu- prous oxide (Cu <sub>2</sub> O)	Cop- per (Cu)	Lactose		
		(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> ½H <sub>2</sub> O)	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> , H <sub>2</sub> O)			(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> ½H <sub>2</sub> O)	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> , H <sub>2</sub> O)
190	168.8	122.7	125.9	129.2	245	217.6	159.4	163.6	167.8
191	169.7	123.4	126.6	129.9	246	218.5	160.1	164.3	168.5
192	170.5	124.1	127.3	130.6	247	219.4	160.7	165.0	169.2
193	171.4	124.7	128.0	131.3	248	220.3	161.4	165.7	169.9
194	172.3	125.4	128.7	132.0	249	221.2	162.1	166.3	170.6
195	173.2	126.1	129.4	132.7	250	222.1	162.7	167.0	171.3
196	174.1	126.7	130.0	133.4	251	222.9	163.4	167.7	172.0
197	175.0	127.4	130.7	134.1	252	223.8	164.1	168.4	172.7
198	175.9	128.1	131.4	134.8	253	224.7	164.7	169.1	173.4
199	176.8	128.7	132.1	135.5	254	225.6	165.4	169.8	174.1
200	177.6	129.4	132.8	136.2	255	226.5	166.1	170.5	174.8
201	178.5	130.0	133.5	136.9	256	227.4	166.6	171.1	175.5
202	179.4	130.7	134.1	137.6	257	228.3	167.4	171.8	176.2
203	180.3	131.4	134.8	138.3	258	229.2	168.1	172.5	176.9
204	181.2	132.0	135.5	139.0	259	230.0	168.8	173.2	177.6
205	182.1	132.7	136.2	139.7	260	230.9	169.4	173.9	178.3
206	183.0	133.4	136.9	140.4	261	231.8	170.1	174.6	179.0
207	183.9	134.0	137.6	141.1	262	232.7	170.8	175.3	179.8
208	184.8	134.7	138.3	141.8	263	233.6	171.4	176.0	180.5
209	185.6	135.4	138.9	142.5	264	234.5	172.1	176.6	181.2
210	186.5	136.0	139.6	143.2	265	235.4	172.8	177.3	181.9
211	187.4	136.7	140.3	143.9	266	236.3	173.5	178.0	182.6
212	188.3	137.4	141.0	144.6	267	237.2	174.1	178.7	183.3
213	189.2	138.0	141.7	145.3	268	238.0	174.8	179.4	184.0
214	190.1	138.7	142.4	146.0	269	238.9	175.5	180.1	184.7
215	191.0	139.4	143.0	146.7	270	239.8	176.1	180.8	185.4
216	191.9	140.0	143.7	147.4	271	240.7	176.8	181.5	186.1
217	192.7	140.7	144.4	148.1	272	241.6	177.5	182.1	186.8
218	193.6	141.4	145.1	148.8	273	242.5	178.1	182.8	187.5
219	194.5	142.0	145.8	149.5	274	243.4	178.8	183.5	188.2
220	195.4	142.7	146.5	150.2	275	244.3	179.5	184.2	188.9
221	196.3	143.4	147.2	150.9	276	245.1	180.2	184.9	189.6
222	197.2	144.0	147.8	151.6	277	246.0	180.8	185.6	190.3
223	198.1	144.7	148.5	152.3	278	246.9	181.5	186.3	191.0
224	199.0	145.4	149.2	153.0	279	247.8	182.2	187.0	191.7
225	199.8	146.0	149.9	153.7	280	248.7	182.8	187.7	192.4
226	200.7	146.7	150.6	154.4	281	249.6	183.5	188.3	193.1
227	201.6	147.4	151.3	155.1	282	250.5	184.2	189.0	193.8
228	202.5	148.0	152.0	155.8	283	251.4	184.8	189.7	194.6
229	203.4	148.7	152.6	156.5	284	252.3	185.5	190.4	195.3
230	204.3	149.4	153.3	157.2	285	253.1	186.2	191.1	196.0
231	205.2	150.0	154.0	157.9	286	254.0	186.9	191.8	196.7
232	206.1	150.7	154.7	158.6	287	254.9	187.5	192.5	197.4
233	207.0	151.4	155.4	159.3	288	255.8	188.2	193.2	198.1
234	207.8	152.0	156.1	160.0	289	256.7	188.9	193.8	198.8
235	208.7	152.7	156.7	160.7	290	257.6	189.5	194.5	199.5
236	209.6	153.4	157.4	161.4	291	258.5	190.2	195.2	200.2
237	210.5	154.0	158.1	162.1	292	259.4	190.9	195.9	200.9
238	211.4	154.7	158.8	162.8	293	260.2	191.5	196.6	201.6
239	212.3	155.4	159.5	163.5	294	261.1	192.2	197.3	202.3
240	213.2	156.1	160.2	164.3	295	262.0	192.9	198.0	203.0
241	214.1	156.7	160.9	165.0	296	262.9	193.6	198.7	203.7
242	214.9	157.4	161.5	165.7	297	263.8	194.2	199.3	204.4
243	215.8	158.1	162.2	166.4	298	264.7	194.9	200.0	205.1
244	216.7	158.7	162.9	167.1	299	265.6	195.6	200.7	205.8

TABLE FOR CALCULATING LACTOSE—Continued

Cu- prous oxide (Cu <sub>2</sub> O)	Cop- per (Cu)	Lactose			Cu- prous oxide (Cu <sub>2</sub> O)	Cop- per (Cu)	Lactose		
		(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> ½H <sub>2</sub> O)	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> , H <sub>2</sub> O)			(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> ½H <sub>2</sub> O)	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> , H <sub>2</sub> O)
300	266.5	196.2	201.4	206.6	355	315.3	233.3	239.4	245.6
301	267.4	196.9	202.1	207.3	356	316.2	233.9	240.1	246.3
302	268.2	197.6	202.8	208.0	357	317.1	234.6	240.8	247.0
303	269.1	198.3	203.5	208.7	358	318.0	235.3	241.5	247.7
304	270.0	198.9	204.2	209.4	359	318.9	236.0	242.2	248.4
305	270.9	199.6	204.9	210.1	360	319.8	236.7	242.9	249.1
306	271.8	200.3	205.5	210.8	361	320.6	237.3	243.6	249.8
307	272.7	201.0	206.2	211.5	362	321.5	238.0	244.3	250.5
308	273.6	201.6	206.9	212.2	363	322.4	238.7	245.0	251.2
309	274.5	202.3	207.6	212.9	364	323.3	239.4	245.7	252.0
310	275.3	203.0	208.3	213.7	365	324.2	240.0	246.4	252.7
311	276.2	203.6	209.0	214.4	366	325.1	240.7	247.0	253.4
312	277.1	204.3	209.7	215.1	367	326.0	241.4	247.7	254.1
313	278.0	205.0	210.4	215.8	368	326.9	242.1	248.4	254.8
314	278.9	205.7	211.1	216.5	369	327.8	242.7	249.1	255.5
315	279.8	206.3	211.8	217.2	370	328.6	243.4	249.8	256.2
316	280.7	207.0	212.5	217.9	371	329.5	244.1	250.5	256.9
317	281.6	207.7	213.1	218.6	372	330.4	244.8	251.2	257.7
318	282.5	208.4	213.8	219.3	373	331.3	245.4	251.9	258.4
319	283.3	209.0	214.5	220.0	374	332.2	246.1	252.6	259.1
320	284.2	209.7	215.2	220.7	375	333.1	246.8	253.3	259.8
321	285.1	210.4	215.9	221.4	376	334.0	247.5	254.0	260.5
322	286.0	211.0	216.6	222.2	377	334.9	248.1	254.7	261.2
323	286.9	211.7	217.3	222.9	378	335.7	248.8	255.4	261.9
324	287.8	212.4	218.0	223.6	379	336.6	249.5	256.1	262.6
325	288.7	213.1	218.7	224.3	380	337.5	250.2	256.8	263.4
326	289.6	213.7	219.4	225.0	381	338.4	250.8	257.5	264.1
327	290.4	214.4	220.1	225.7	382	339.3	251.5	258.1	264.8
328	291.3	215.1	220.7	226.4	383	340.2	252.2	258.8	265.5
329	292.2	215.8	221.4	227.1	384	341.1	252.9	259.5	266.2
330	293.1	216.4	222.1	227.8	385	342.0	253.6	260.2	266.9
331	294.0	217.1	222.8	228.5	386	342.9	254.2	260.9	267.6
332	294.9	217.8	223.5	229.2	387	343.7	254.9	261.6	268.3
333	295.8	218.4	224.2	230.0	388	344.6	255.6	262.3	269.0
334	296.7	219.1	224.9	230.7	389	345.5	256.3	263.0	269.8
335	297.6	219.8	225.6	231.4	390	346.4	256.9	263.7	270.5
336	298.4	220.5	226.3	232.1	391	347.3	257.6	264.4	271.2
337	299.3	221.1	227.0	232.8	392	348.2	258.3	265.1	271.9
338	300.2	221.8	227.7	233.5	393	349.1	259.0	265.8	272.6
339	301.1	222.5	228.3	234.2	394	350.0	259.6	266.5	273.3
340	302.0	223.2	229.0	234.9	395	350.8	260.3	267.2	274.0
341	302.9	223.8	229.7	235.6	396	351.7	261.0	267.9	274.7
342	303.8	224.5	230.4	236.3	397	352.6	261.7	268.6	275.5
343	304.7	225.2	231.1	237.0	398	353.5	262.3	269.3	276.2
344	305.5	225.9	231.8	237.8	399	354.4	263.0	269.9	276.9
345	306.4	226.5	232.5	238.5	400	355.3	263.7	270.6	277.6
346	307.3	227.2	233.2	239.2	401	356.2	264.4	271.3	278.3
347	308.2	227.9	233.9	239.9	402	357.1	265.0	272.0	279.0
348	309.1	228.5	234.6	240.6	403	358.0	265.7	272.7	279.7
349	310.0	229.2	235.3	241.3	404	358.8	266.4	273.4	280.4
350	310.9	229.9	235.9	242.0	405	359.7	267.1	274.1	281.1
351	311.8	230.6	236.6	242.7	406	360.6	267.8	274.8	281.9
352	312.7	231.2	237.3	243.4	407	361.5	268.4	275.5	282.6
353	313.5	231.9	238.0	244.1	408	362.4	269.1	276.2	283.3
354	314.4	232.6	238.7	244.8	409	363.3	269.8	276.9	284.0

TABLE FOR CALCULATING LACTOSE—Continued

Cu- prous oxide (Cu <sub>2</sub> O)	Cop- per (Cu)	Lactose			Cu- prous oxide (Cu <sub>2</sub> O)	Cop- per (Cu)	Lactose		
		(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> ½ H <sub>2</sub> O)	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> , H <sub>2</sub> O)			(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> )	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> ½ H <sub>2</sub> O)	(C <sub>12</sub> H <sub>22</sub> O <sub>11</sub> , H <sub>2</sub> O)
410	364.2	270.5	277.6	284.7	450	399.7	297.6	305.5	313.3
411	365.1	271.2	278.3	285.4	451	400.6	298.3	306.2	314.0
412	365.9	271.8	279.0	286.2	452	401.5	299.0	306.9	314.7
413	366.8	272.5	279.7	286.9	453	402.4	299.7	307.6	315.5
414	367.7	273.2	280.4	287.6	454	403.3	300.4	308.3	316.2
415	368.6	273.9	281.1	288.3	455	404.1	301.1	309.0	316.9
416	369.5	274.6	281.8	289.0	456	405.0	301.7	309.7	317.6
417	370.4	275.2	282.5	289.7	457	405.9	302.4	310.4	318.3
418	371.3	275.9	283.2	290.4	458	406.8	303.1	311.1	319.0
419	372.2	276.6	283.9	291.2	459	407.7	303.8	311.8	319.8
420	373.1	277.3	284.6	291.9	460	408.6	304.5	312.5	320.5
421	373.9	277.9	285.3	292.6	461	409.5	305.1	313.2	321.2
422	374.8	278.6	286.0	293.3	462	410.4	305.8	313.9	321.9
423	375.7	279.3	286.7	294.0	463	411.2	306.5	314.6	322.6
424	376.6	280.0	287.4	294.7	464	412.1	307.2	315.3	323.4
425	377.5	280.7	288.1	295.4	465	413.0	307.9	316.0	324.1
426	378.4	281.3	288.8	296.2	466	413.9	308.6	316.7	324.8
427	379.3	282.0	289.4	296.9	467	414.8	309.2	317.4	325.5
428	380.2	282.7	290.1	297.6	468	415.7	309.9	318.1	326.2
429	381.0	283.4	290.8	298.3	469	416.6	310.6	318.8	326.9
430	381.9	284.1	291.5	299.0	470	417.5	311.3	319.5	327.7
431	382.8	284.7	292.2	299.7	471	418.4	312.0	320.2	328.4
432	383.7	285.4	292.9	300.5	472	419.2	312.6	320.9	329.1
433	384.6	286.1	293.6	301.2	473	420.1	313.3	321.6	329.8
434	385.5	286.8	294.3	301.9	474	421.0	314.0	322.3	330.5
435	386.4	287.5	295.0	302.6	475	421.9	314.7	323.0	331.3
436	387.3	288.1	295.7	303.3	476	422.8	315.4	323.7	332.0
437	388.2	288.8	296.4	304.0	477	423.7	316.1	324.4	332.7
438	389.0	289.5	297.1	304.7	478	424.6	316.7	325.1	333.4
439	389.9	290.2	297.8	305.5	479	425.5	317.4	325.8	334.1
440	390.8	290.9	298.5	306.2	481	426.3	318.1	326.5	334.8
441	391.7	291.5	299.2	306.9	481	427.2	318.8	327.2	335.6
442	392.6	292.2	299.9	307.6	482	428.1	319.5	327.9	336.3
443	393.5	292.9	300.6	308.3	483	429.0	320.1	328.6	337.0
444	394.4	293.6	301.3	309.0	484	429.9	320.8	329.3	337.7
					485	430.8	321.5	330.0	338.4
445	395.3	294.2	302.0	309.7	486	431.7	322.2	330.7	339.1
446	396.1	294.9	302.7	310.5	487	432.6	322.9	331.4	339.9
447	397.0	295.6	303.4	311.2	488	433.5	323.6	332.1	340.6
448	397.9	296.3	304.1	311.9	489	434.3	324.2	332.8	341.3
449	398.8	297.0	304.8	312.6	490	435.2	324.9	333.5	342.0

**Estimation of Lactose by Optical Methods.**—Lactose may be estimated by observing the optical activity of its solution. To apply this method to milk, it is first necessary to prepare a clear whey free from other optically active substances. Precipitation with basic lead acetate, as has been shown by Wiley, does not remove completely the lœvrotatory protein matters; he has proposed two alternative mercurial reagents. His method, which has been adopted by the A. O. A. C., is as follows:

(a) **Acid Mercuric Nitrate.**—Dissolve mercury in double its weight of nitric acid, sp. gr. 1.42, and dilute with an equal volume of water. 1 c.c. of this reagent is sufficient for the quantities of milk mentioned below. Larger quantities may be used without affecting the results of polarisation.

(b) **Mercuric Iodide with Acetic Acid.**—Mix 33.2 grm. of potassium iodide, 13.5 grm. of mercuric chloride, 20 c.c. of glacial acetic acid, and 640 c.c. of water.

**Estimation.**—The milk should be at a constant temperature, and its sp. gr. ascertained with a delicate hydrometer. When greater accuracy is required, a pycnometer is used.

The quantities of the milk measured for polarisation differ with the sp. gr. of the milk.

The volume to be measured will be found in the following table which is based upon twice the normal weight of lactose (32.9 grm. per 100 c.c.) for the Ventzke sugar scale.

VOLUMES OF MILK CORRESPONDING TO A LACTOSE  
DOUBLE NORMAL WEIGHT<sup>1</sup>

Sp. gr. of milk	Volume of milk for a lactose double normal weight (Ventzke scale), c.c.	Sp. gr. of milk	Volume of milk for a lactose double normal weight (Ventzke scale), c.c.
1.024	64.25	1.030	63.90
1.025	64.20	1.031	63.80
1.026	64.15	1.032	63.75
1.027	64.05	1.033	63.70
1.028	64.00	1.034	63.65
1.029	63.95	1.035	63.55
.....	.....	1.036	63.50

Place the quantity of milk indicated in the table in a flask graduated at 102.6 c.c. for a Ventzke polariscope. Add 1 c.c. of mercuric nitrate solution or 30 c.c. of mercuric iodide solution (an excess of those reagents does no harm), fill to the mark, agitate, filter through a dry filter, and polarise. It is not necessary to heat before polarising. In case a 200 mm. tube is used, divide the polariscope reading

<sup>1</sup> Browne, *Handbook of Sugar Analysis*, 1912, 252.

by 2 (or if a 400 mm. tube is used, by 4) to obtain the percentage of lactose in the sample.

H. D. Richmond (*Dairy Analysis*) corrects for the sp. gr. and percentage of fat of the milk as follows: to 50 c.c. of milk a quantity of water is added equal in c.c. to the sum of—

(a) The degrees of gravity divided by 20.

(b) The percentage of fat divided by 1.8.

(c) A factor to convert scale readings into percentages of anhydrous sugar. This is 5.43 c.c. if the scale is in angular degrees and a 200 mm. tube is used.

1.5 c.c. of Wiley's acid mercuric nitrate solution is then added and after violent shaking the solution is filtered through a dry filter.

Richmond gives the following example: a milk has a sp. gr. of 1.032 and 3.60% of fat. (a) is  $32.0/20 = 1.6$  c.c., (b) =  $3.6/1.8 = 2.0$  c.c., (c) = 5.43 c.c. The water added is, therefore,  $1.6 + 2.0 + 5.43 = 9.03$  c.c.

According to Richmond (*Analyst*, 1910, **35**, 516), the acid mercuric nitrate reagent does not remove the laevorotatory proteins quite completely, and he recommends that the milk, after treatment with acid mercuric nitrate in the usual way, should be treated with 5% of phosphotungstic acid and 5% of sulphuric acid (1:1). The solution is then filtered and polarised, and the polarisation reading multiplied by 1.1.

In the case of human milk, clarification is more difficult and a turbid filtrate is obtained with ordinary precipitating agents. Thebault (*J. Pharm.*, (6), **4**, 5) uses a solution of picric acid 10 grm. in 1000 c.c. and acetic acid 25 c.c. in 1000 c.c.

**Estimation of Sugars in Condensed Milk.**—To estimate lactose in condensed milk, the proteins must be removed by precipitation and the reducing sugar in the whey determined. The cane sugar present may be approximately estimated by difference; that is, subtracting the sum of the other ingredients from the total solids. The direct methods are all based on the removal of the proteins and determination of the cupric reducing or optical rotatory power before and after inversion. This must be effected by a mild agent, such as citric acid or invertase, since mineral acids will also hydrolyse the lactose.

Stokes and Bodmer (*Analyst*, 1885, **10**, 62) coagulate the milk by the addition of 1% of citric acid without heating, dilute, filter, and estimate the reducing power of the clear filtrate. To another portion

of the filtrate a further 1% of citric acid is added and the solution boiled for 10 minutes, according to the authors, or better, for at least 30 minutes, according to Watts and Tempany (*ibid.*, 1905, 30, 119).

The solution is cooled, neutralised and the reducing power again determined. The increase is due to the invert sugar formed from the sucrose.

Leffmann and Beam use invertase for inversion. The proteins are precipitated by mercuric nitrate and the clear whey polarised. In a portion of the filtrate, the acid is carefully neutralised, a drop of acetic acid is added and a small quantity of invertase along with a few drops of an antiseptic. The whole is incubated at 35° to 40° for 24 hours. After the action, the proteins are precipitated by alumina cream and the liquid made to known volume and again polarised. The difference between the two readings is calculated as sucrose.

Bigelow and McElroy (*J. Amer. Chem. Soc.*, 1893, 15, 668) propose the following routine method for the estimation of the sugars, including invert sugar, in condensed milk. The solutions used are:

*Acid Mercuric Iodide.*—See page 462. *Alumina Cream.*—See page 374.

The entire contents of a can are transferred to a porcelain dish and thoroughly mixed. A number of portions about 25 grm. each are weighed carefully in 100 c.c. flasks. Water is added to two of the portions and the solutions boiled. The flasks are cooled, their contents clarified by means of a small amount of each of the above solutions, made up to the mark, shaken, filtered, and the polarimetric reading noted. Other weighed portions are heated in the water-bath to 55°, one-half of a cake of compressed yeast added to each flask, and the temperature maintained at 55° for 5 hours. The solutions are then clarified as before, cooled to room temperature, made up to 100 c.c., mixed, filtered, and the polarimetric reading taken. The amount of sucrose is estimated by the formula on page 463. Correction for the volume of precipitated solids may be made by the double-dilution method. The total reducing sugar is estimated by one of the reducing methods on one of the weighed portions of the original material, and if the sum of it reckoned as milk sugar and the amount of sucrose estimated by the inversion method would give a polarisation equal to the direct reading of both sugars before

inversion, no invert sugar is present. If the amount of reducing sugar seems too great, the milk sugar must be reestimated as follows: 250 grm. of the sample are dissolved in water, the solution boiled, cooled to 80°, a solution of about 4 grm. of glacial phosphoric acid added, the mixture kept at 80° for a few minutes, then cooled to room temperature, made up to a definite volume, mixed, and filtered. It may be assumed that the precipitate produced by the phosphoric acid is equal in volume to that produced by the acid mercuric iodide. Potassium hydroxide is then added in amount not quite sufficient to neutralise the acid, and sufficient water to make up for the solids precipitated by the acid. The mixture is then filtered and the filtrate measured in portions of 100 c.c. into 200 c.c. flasks. A solution containing 20 mg. of potassium fluoride and half a cake of compressed yeast are added to each flask, and the mixture allowed to stand for 10 days at a temperature of from 25° to 30°. The invert sugar and sucrose are fermented and removed whilst the milk sugar is unaffected. The flasks are filled to the mark, shaken, and the milk sugar estimated by either reduction or the polariscope. The amount of copper reduced by the milk sugar and invert sugar, less the equivalent of milk sugar remaining after fermentation, is due to invert sugar.

C. B. Cochran (*J. Amer. Chem. Soc.*, 1907, **29**, 555) makes use of Wiley's acid mercuric nitrate solution to invert sucrose in the analysis of sweetened condensed milk. He finds this inverts sucrose only very slowly at temperatures below 15°. 50 c.c. of the solution to be inverted (containing 3 c.c. of mercuric solution per 100 c.c.) are polarised as soon as possible after the solution has been mixed at 15° and then heated in boiling water for 7 minutes and again polarised. The sucrose-content in the case of normal solutions is given by the formula:

$$\text{Sucrose} = \frac{100D}{132.68 - 0.5t} \text{ where } D \text{ is the difference in polarisation}$$

before and after inversion and  $t$  = temperature above 20°.

The official A. O. A. C. method for estimating lactose in sweetened condensed milk is as follows: 100 c.c. of a 20% solution of the sample are diluted to about 200 c.c. in a 250 c.c. flask, treated with 6 c.c. of a solution of copper sulphate of the strength given for Soxhlet's modification of Fehling's solution, and made up to the mark. The solution is filtered through a dry filter, and the lactose estimated

gravimetrically, e. g., by Munson and Walker's method (see page 401).

To detect sucrose in condensed milk or milk sugar, Leffmann applies the sesame oil test. 1 c.c. of sesame oil, 1 c.c. of concentrated hydrochloric acid and 0.5 gm. of the sample are shaken together. The characteristic crimson colouration will be formed within half an hour.

This test has been found to be satisfactory and is better than that given in the United States Pharmacopœia which depends on carbonisation of the sucrose by strong sulphuric acid. A rapid test for sucrose in milk and cream consists in boiling a mixture of 15 c.c. of milk, 0.1 gm. of resorcinol and 1 c.c. of concentrated hydrochloric acid. Sucrose gives a fine red coloration, pure milk remains almost unchanged. Elsdon (*Analyst*, 1918, 43, 292) modifies this test by evaporating 5 drops of a mixture of 1 c.c. of 3 N-hydrochloric acid, 0.5 gm. of resorcinol and 15 c.c. of the milk-to dryness on the water-bath in a depression of a white spotting tile and states that as little as 0.02% of sucrose in milk can thus be detected.

## MONO-SACCHARIDES

Mono-saccharides of commercial importance are the hexoses  $C_6H_{12}O_6$ , more generally termed glucoses, and the pentoses  $C_5H_{10}O_5$ .

**Glucoses.**—Their generic and specific characters are contained in the table on page 357. Since they are very closely related in structure, differing indeed, with the exception of lævulose, only in the space arrangement of the groups in their molecule, their chemical properties are very similar. As a class they are (1) not susceptible of inversion; (2) are readily and directly fermented by yeast; (3) are decomposed by alkalis; (4) are readily oxidised by alkaline solutions of copper.

### Dextrose.—d-Glucose.

This is produced from various poly-saccharides and glucosides by hydrolysis with acids or enzymes and also from cellulose materials. It is found ready formed in various fruits, the proportion in grapes being as high as 15%. It exists in two isomeric modifications which are mutually transformed into one another in solution.

The  $\alpha$ -isomeride crystallises from aqueous solutions at  $30^\circ$ – $35^\circ$  as the hydrate,  $C_6H_{12}O_6 \cdot H_2O$ . The anhydrous  $\alpha$ -isomeride may be obtained by crystallisation from 90% ethyl alcohol or from methyl

alcohol or from a mixture of 1000 c.c. of glacial acetic acid and 500 grm. of dextrose dissolved in 250 c.c. of water (Hudson and Dale, *J. Amer. Chem. Soc.*, 1917, 39, 320); it has an initial rotatory power of  $[\alpha]_D = +110^\circ$  and melts at  $146^\circ$ – $147^\circ$ .

The  $\beta$ -isomeride may be obtained by heating the  $\alpha$ -isomeride to  $105^\circ$ – $110^\circ$  for 12 hours or by crystallisation from a mixture of a solution of 500 grm. of dextrose in 50 c.c. of water and 600 c.c. of glacial acetic acid and recrystallisation of the product by dissolving it in an equal amount of ice-cold water and adding alcohol (*loc. cit.*); it has an initial rotatory power of  $[\alpha]_D = +19^\circ$ .

Both the  $\alpha$ - and  $\beta$ -isomeride exhibit mutarotation in aqueous solution, each being converted into the equilibrium mixture of  $[\alpha]_D = +52.5^\circ$  in the course of some hours at the ordinary temperature, or immediately on heating or on addition of a trace of alkali.

According to Browne (*J. Ind. Eng. Chem.*, 1910, 2, 526) the "normal" weight of dextrose (*i. e.*, that quantity which, dissolved in 100 metric c.c. and polarised at  $20^\circ$  in a 200 mm. tube, reads exactly  $100^\circ$  V.) is 32.25 grm., and the percentage of dextrose,  $D$ , corresponding to any scale reading,  $S$ , is almost exactly expressed by the formula

$$D = S + 0.02 S - 0.0002 S^2.$$

Characteristic of dextrose (and glucuronic acid) is the formation of saccharic acid on oxidation. 5 grm. of the sugar are heated at the temperature of the water-bath with 30 c.c. nitric acid (sp. gr. 1.15) to a thick syrup. This is taken up with water and again evaporated to remove excess of acid, neutralised with potassium carbonate and a few drops of acetic acid added, when the potassium saccharate crystallises out on standing.

Characteristic also is the very insoluble phenylosazone, m. p.  $205$ – $210^\circ$ . Tutin gives this as  $217^\circ$  on recrystallisation from pyridine (*Proc. Chem. Soc.*, 1907, 23, 50), but the older value is the correct one (Fischer, *Ber.*, 1908, 41, 73).

Dextrose diphenylhydrazone, m. p.  $161^\circ$ , and the methylphenylhydrazone, m. p.  $130^\circ$ , may be used to identify or detect dextrose, particularly in presence of pentoses.

#### **Lævulose.—d-Fructose.—Fruit Sugar.**

Lævulose differs from dextrose in containing a ketonic grouping. It occurs together with dextrose in honey and many fruits and is pro-

duced together with dextrose on hydrolysis of sucrose or with dextrose and other sugars on hydrolysis of some poly-saccharides. Inulin, a poly-saccharide found in dahlia tubers, yields lævulose alone when hydrolysed. No glucoside containing lævulose has been as yet isolated. The principal physical and chemical properties of lævulose are contained in the table on page 357. Its behaviour is very similar to that of dextrose, but it is not readily crystallisable. It yields glycollic acid when oxidised with bromine water and not gluconic acid.

Lævulose has a value  $[\alpha]_D^{20} = -92^\circ$ , which value decreases  $0.6385^\circ$  for each rise of  $1^\circ$  in the temperature. The rotation at  $87.2^\circ$  is  $-52.7^\circ$ ; that is equal, but opposite, to that of dextrose at the same temperature.

This change in the optical activity of lævulose affords a means of estimating it in presence of other sugars. The solution is examined in a jacketed polarimeter tube provided with a thermometer and the rotation noted at two temperatures as far apart as possible. It is advisable to use fairly strong solutions. To calculate the number of grams of lævulose in 100 c.c. of solution, the difference between the two temperatures is multiplied by 1.277 and this product divided into 100 times the alteration in rotation measured in circular degrees in a 200 mm. tube by sodium light. In view of the fact that the rotatory power of other sugars likewise differs with the temperature, this method can hardly claim any great accuracy.

The respective reducing actions of dextrose and lævulose on Fehling's solution are usually assumed to be identical. According to Soxhlet, however, the reducing action of the former is sensibly greater than that of the latter. Allihn states that the reducing actions of dextrose and lævulose are identical if care be taken to continue the boiling of the solution for half an hour.

Lævulose, on account of its ketonic nature, reduces alkaline copper solutions more rapidly than do other sugars and at a lower temperature and this property may be made use of for its estimation. The subject has been fully investigated by J. Pieraerts (*Bull. Assoc. Chim. Sucr.*, 1908, **25**, 830), who has made comparative trials of a number of reagents. Excellent results were obtained with a cuproglycocol solution (6 grm. of cupric hydroxide, 12 grm. of glycocol and 50 grm. of potassium carbonate dissolved in water and made up to 1 litre) which is reduced by lævulose at normal temperature in

12 hours, but totally unaffected by other hexose or pentose sugars in 24 hours. For the determination of lævulose in commercial preparations the following general method is prescribed. 20 to 25 grm. of material are dissolved in 150 to 200 c.c. of cold water, clarified with basic lead acetate solution and the excess of lead removed by saturated sodium sulphate solution. After half an hour, the solution is filtered and diluted so as to contain 5% reducing sugar and tested for lævulose as above.

Other suitable reagents are: alkaline cupric hydroxide (100 grm. of potassium carbonate, 50 or 75 grm. of potassium hydrogen carbonate and 6 grm. of cupric hydroxide in 1000 c.c.). With this, lævulose may be detected with certainty even when much pentose is present. The action is continued for 3 hours at the ordinary temperature or, in the absence of pentose, for 1 hour at 30°.

1 grm. lævulose in 1% solution reduces 508.5 c.c. of Knapp's and 449.5 c.c. of Sachsse's mercury reagents. The reducing action on Knapp's solution is about the same as that of dextrose, but dextrose has a considerably weaker reducing action on Sachsse's solution, equal amounts of dextrose and lævulose reducing 100 c.c. and 148.6 c.c. respectively.

According to Neuberg, the methylphenylosazone of lævulose is very characteristic. It forms long yellow needles, m. p. 158° to 160°.

**Invert Sugar.**—Invert sugar exists largely in honey, molasses, and many fruits. It is a mixture of equivalent proportions of dextrose and lævulose, produced by the action of heat, some enzymes, acids, salts or other agents on sucrose. The conditions most favourable for its formation have already been described.

Invert sugar is usually a syrup having a sweeter taste than cane sugar. In its chemical reactions and optical properties it behaves strictly as a mixture of dextrose and lævulose. Invert sugar is now made largely for brewers' use, being sold under the names of "invert" or "inverse sugar," "saccharum," "malt-saccharum," and other trade names. Starch-sugar and cane sugar are often added. The analysis of such products may be effected in the same manner as that of honey, but it is generally sufficient to estimate the sugar by Fehling's solution before and after inversion. These estimations give the data for calculating the cane or uninverted sugar and the total invert sugar without distinguishing between the dextrose and lævulose.

The following method for estimating the sugars and unfermentable matter in invert sugar is due to G. H. Morris (*J. Inst. Brew.*, 1898, 4, 162):

10 c.c. of the 10% solution of the sample used for the estimation of water by the solution density method (see page 363) are diluted to 50 c.c., and 10 c.c. of the diluted solution (= 0.2 grm. of sample) are used for the estimation of reducing power by the method of Brown, Morris and Millar (see page 397). The weight of cupric oxide or copper thus obtained, multiplied by 500, represents the amount reduced by 100 grm. of the sample. A further portion of the 10% solution of the sample is used for polarimetric reading in a 200 mm. tube of the degrees Ventzke. Since the copper reducing power of the sample is affected by the unfermentable matter and the polarimeter reading is affected both by the unfermentable matter and by sucrose if present, the influence of these constituents must be determined. The sucrose is estimated by double polarisation, using pressed yeast as hydrolyst. For the unfermentable matter, 50 c.c. of the 10% solution of the sample are sterilised by boiling for a few minutes, cooled and fermented at about 24° with 2 grm. of washed, pressed yeast for about 48 hours or longer if necessary; the fermented liquid is treated with a little alumina cream, made up to 100 c.c., and filtered, the filtrate being used for the estimation of reducing and rotatory powers. The data thus obtained serve for calculating the percentages of dextrose and lævulose as follows:

From the amount of cupric oxide reduced by 100 grm. of the sample is deducted the amount due to the reducing power of the unfermentable matter in 100 grm., the remainder,  $a$ , representing the amount reduced by the dextrose and lævulose in 100 grm. Reference is now made to Brown, Morris and Millar's table (page 399) for the weight of cupric oxide,  $m$ , corresponding to 1 grm. of dextrose (column 4), and for the weight of cupric oxide,  $n$ , corresponding to 1 grm. of lævulose (column 7); as will be seen from the table, the values of  $m$  and  $n$  vary with the amount of copper or cupric oxide obtained in the estimation. Then if  $D$  be the percentage of dextrose and  $L$  that of lævulose

$$mD + nL = a \quad (1)$$

Again, the polarimetric reading in degrees Ventzke of the 10% solution is corrected for the rotation of the sucrose and unfermentable

matter and the corrected reading in  $^{\circ}\text{V}$  is converted to  $[\alpha]_D$  of the sugars (*i. e.*, dextrose and lævulose) from the relation

$$[\alpha]_D = \frac{^{\circ}\text{V} \times 10}{2} \times 0.346^1$$

If the value of  $[\alpha]_D$  thus obtained is denoted,  $b$ , we have

$$52.7D - 92.0L = b \times 100 \quad (2)$$

the values 52.7 and  $-92.0$  being the specific rotations of dextrose and lævulose at  $20^{\circ}$ , the polarisation temperature. The values of  $D$  and  $L$  are calculated from equations (1) and (2).

The percentage of unfermentable matter is usually found by deducting the sum of the percentages of dextrose, lævulose, sucrose, ash, water and albuminoid matter from 100.

*Analyses of Invert Sugar* (Typical).—From Moritz and Morris' "*Text-book of the Science of Brewing*."

	Good	INFERIOR
Invert sugar,	75.23	60.53
Cane sugar,	0.95	8.56
Ash,	1.16	5.53
Proteins,	0.78	1.89
Water,	19.23	13.77
Other matters,	2.65	9.72
	100.00	100.00

**Galactose.**—Galactose is formed, together with dextrose, by the hydrolysis of milk sugar; it occurs in some glucosides, most gums and many plant products. It is much less sweet than sucrose, yields dulcitol on reduction and galactonic acid when oxidised with bromine water.

It has a value of  $[\alpha]_D = 81.27^{\circ}$  for a 10% solution at  $15^{\circ}$  and the rotation at temperature  $t$  and concentration  $c$  may be calculated from the formula:

$$[\alpha]_D = 83.137 + 0.199c - (0.276 - 0.0025c)t$$

It exhibits mutarotation. Other physical data will be found on page 389.

Characteristic is the  $\alpha$ -methylphenylhydrazone, which forms colourless needles, m. p.  $180^{\circ}$ , and is sparingly soluble in water and abso-

<sup>1</sup> For most purposes the factor 0.346 used for converting degrees Ventzkes to  $[\alpha]_D$  is sufficiently accurate; more exact values for various sugars are given on page 372.

lute alcohol. According to Van der Haar (*Rec. Trav. Chim. Pays-Bas*, 1917, **37**, 108), the *o*-tolylhydrazone, which forms colourless needles, m. p.  $176^{\circ}$ , serves to characterise galactose in presence of various other aldoses. It may be separated from dextrose by such yeasts as *S. apiculatus* and *S. Ludwigii*, which ferment dextrose, but not galactose (see page 471). A fermentation test with one of these yeasts affords a very delicate means of detecting the presence of small quantities of dextrose in a sample of galactose. Commercial galactose invariably contains a small percentage of dextrose.

Characteristic of galactose and also of lactose is the formation of mucic acid on oxidation with nitric acid.

For the preparation of mucic acid the sugar should be slowly evaporated on the water-bath with about 4 times its weight of nitric acid of 1.27 sp. gr. or 10 times its weight of acid of 1.15 sp. gr. until a thick syrup is formed. This is diluted with a little water and allowed to stand for some hours. Mucic and oxalic acid will crystallise out and may be separated by warm alcohol in which only the oxalic acid dissolves.

Tollens (*Annalen*, 1885, **227**, 223) applies this method quantitatively as follows: 5 grm. of the dry sugar are placed in a beaker 6 cm. in diameter with 50 c.c. of nitric acid, sp. gr. 1.15, and evaporated at the heat of the water-bath to one-third of the volume. When cold, 0.5 grm. of pure mucic acid and 100 c.c. of water are added. After 1 or, better, 2 days' standing with occasional stirring the crystallised solid is collected on a weighed filter, washed twice with 5 c.c. of cold water, dried at  $100^{\circ}$  and weighed. After subtraction of the 0.5 grm. of mucic acid added to facilitate crystallisation, 77.4 parts of mucic acid correspond to 100 parts of galactose.

Should impurities, such as cellulose or calcium salts, remain after washing, the precipitate and filter-paper are warmed with a solution of ammonium carbonate, filtered and the filtrate evaporated nearly to dryness in a dish. The mucic acid is precipitated on the addition of nitric acid and may be collected and weighed.

This method has proved useful in the estimation of the galactose-yielding groups in complex carbohydrates; in presence of large quantities of foreign, organic matter, however, the crystallisation of mucic acid may be hindered or altogether prevented.

Tables showing the quantities of galactose corresponding to the weights of mucic acid obtained under specified conditions from pure

galactose and mixtures of galactose and sucrose have been worked out by Van der Haar (*Chem. Weekblad*, 1916, 13, 1204; *Analyst*, 1917, 42, 23).

**Commercial Glucose—Starch-sugar.**—Several partially or fully converted starch-sugars of which dextrose is the leading constituent are sold under the name of glucose, starch-sugar and a variety of other more fanciful appellations. Commercial glucoses are very largely used as substitutes for other carbohydrates, *e. g.*, for malt in brewing, in honey and for the manufacture of factitious wine.

Starch-glucose occurs in commerce in several forms, ranging from the condition of pure anhydrous dextrose, through inferior kinds of solid sugar, to the condition of a thick, syrupy liquid resembling glycerol, which contains a large proportion of dextrin.

In America, the term "glucose" is restricted to the syrupy preparations, the solid products being distinguished as "grape sugar." The following grades are recognised:

**Liquid Varieties.**—Glucose, mixing glucose, mixing syrup, corn syrup, jelly glucose and confectioners' crystal glucose.

**Solid Varieties.**—Solid grape sugar, clipped grape sugar, granulated grape sugar, powdered grape sugar, confectioners' grape sugar, brewers' grape sugar.

The United States standard of purity states that glucose, mixing glucose or confectioners' glucose has a sp. gr. at 100° F. of from 41° Bé. (21% water) to 45° Bé. (14% water) and contains not more than 1% ash on a basis of 41° Bé.

Commercial starch-glucose is produced by the action of dilute acid on starch or starchy matter or occasionally woody fibre. In America it appears to be wholly made from maize starch, and is often termed "corn syrup," but in Europe rice and potato starches are frequently used.

As a rule, in the United States, hydrochloric acid is the converting agent, the proportion employed ranging in practice from 1 to 3% according to the kind of product desired and the details of the subsequent manipulation. The starch, or amylaceous substance, is either boiled with the acid and water in an open tank or heated with it in strong copper cylinders under high pressure. If the first method be adopted and the process arrested as soon as a cold sample of the liquid ceases to give a blue colour with iodine, the product contains a large proportion of dextrin; but if high pressure be employed and the

action pushed farther, dextrose is the chief product. In either mode of operating, maltose and, very commonly, other products are formed in addition to dextrose and dextrin. The acid is next neutralised, the liquid decolorised, if necessary, by animal charcoal and evaporated *in vacuo* till it acquires a sp. gr. of 1400 to 1420.

When sulphuric acid is used as the converting agent the product retains a considerable quantity of dissolved calcium sulphate. Oxalic acid is also sometimes used. As a result of the method of manufacture, inferior qualities of glucose may contain sulphurous or sulphuric acid, calcium sulphate or chlorides, arsenic and lead compounds. Arsenic may be detected by Reinsch's test or by the methods referred to under "Malt;" for lead see p. 195. The mineral matter may be estimated by the weight and composition of the ash, which should not in a good product exceed 1% and should be almost wholly free from iron if the glucose is to be used for brewing. Sometimes the calcium sulphate is removed by treating the concentrated solution with barium oxalate. The amount of free acid is estimated by titration with standard alkali and phenolphthalein; many specimens possess normally a slightly acid reaction, probably due to acid phosphates. Water may be estimated by one of the methods given on page 442, a high temperature being carefully avoided. Nitrogenous matter is conveniently estimated by the Kjeldahl process or by ignition with soda-lime.

Most commercial starch-sugars contain, in addition to dextrose and dextrin, maltose and a notable percentage of unfermentable carbohydrates, apparently produced by over-treatment with acid. The term "gallisin" is applied to these.

The majority of the published analyses of glucose products have failed to take all these constituents into account or are based on faulty methods of analysis. In consequence, much confusion exists on this particular subject.

Gallisin, as hitherto obtained, is not a definite compound, and it appears advisable only to retain the term as synonymous with unfermentable matter. The whole question of the structure of starch, the nature of the various dextrans and of isomaltose still remains a vexing question in carbohydrate chemistry and the utmost confusion exists as regards the subject. (The reader is referred to Ling's article on Starch in Sykes' "*Text-book of Brewing*.")

The following analyses of commercial glucoses, quoted by W. G. Valentin (*J. Soc. Arts*, 1875, 6, 24, 404) are among the most complete and probably most reliable hitherto published:

	No. 1	No. 2	No. 3	No. 4	No. 5
Dextrose.....	80.00	58.85	67.44	63.42	61.46
Maltose.....	none	14.11	10.96	13.50	13.20
Dextrin.....	none	1.70	none	none	none
Unfermentable carbohydrates with a little protein matter....	8.20	9.38	4.30	8.40	8.60
Mineral matter.....	1.30	1.40	1.60	1.50	1.60
Water.....	10.50	14.56	15.70	13.18	15.20
	100.00	100.00	100.00	100.00	100.06
Total solid matter.....	89.50	85.44	84.30	86.82	84.80
Matter of use to the brewer....	80.00	74.66	78.40	76.92	74.60

No. 1 was somewhat brown, very hard, and of English manufacture. No. 2 was pale straw-coloured, softish, French. No. 3, whitish, somewhat hard, English. No. 4, whitish, somewhat hard, German. No. 5, white, somewhat hard, German.

The following analyses are by I. Steiner (*Dingler's polyt. J.*, 1879, 233, 262):

	No. 1	No. 2	No. 3	No. 4
Dextrose.....	45.40	26.50	76.00	
Maltose.....	28.00	40.30	5.00	42.60
Dextrin.....	9.30	15.90	....	39.80
Unfermentable carbohydrates.....	1.50	7.00	5.30	8.90
Proteins.....	traces	1.80	0.20	
Free acid (as H <sub>2</sub> SO <sub>4</sub> ).....	0.08	0.03	0.05	
Mineral matter.....	0.30	2.50	0.40	1.10
Water.....	15.50	6.00	13.30	7.60
	100.08	100.03	100.25	100.00
Total solid matter.....	84.42	93.97	86.65	92.40
Matter of use to the brewer .....	82.70	82.70	81.00	82.40

No. 1 was of German origin, white and soft. The other samples were English, and made from maize without previous separation of the starch.

These analyses are unusually elaborate, and for commercial purposes there is no occasion to enter so much into detail. Many analysts limit their statements to the proportions of water, ash, dextrin, and glucose, ignoring the maltose altogether. This practice is very objectionable as, in an analysis so stated not only is the maltose classed as dextrose, but the amount of dextrin is also seriously in error. Nevertheless, the cupric reducing power of the sample is a character of considerable value for the commercial classification of a glucose or for analysing a sample during the process of conversion, provided its true meaning be not misinterpreted. Taken together with the specific rotatory power of a sample, and the percentage of ash and water, it often affords ample information for commercial purposes.

Morris' method for the analysis of invert sugar (see page 470) may also be employed for the estimation of dextrose and maltose in starch-sugar. The estimation of sucrose may be omitted in this case, since this sugar is not present in starch-sugar. The percentage of maltose being denoted by  $M$  and the weight of cupric oxide corresponding to 1 gram. of maltose by  $p$  (see Brown, Morris and Millar's table on page 400) the equations are modified as follows:

$$mD + pM = a \quad (1)$$

$$52.7D + 138.0M = b \times 100 \quad (2)$$

*Estimation of Commercial Glucose in Admixture with Invert Sugar.*—It is manifestly impossible to estimate with absolute accuracy the amount of commercial glucose added as an adulterant by reason of the differing amounts of dextrose, maltose and dextrin present in commercial glucose.

When the amount of invert sugar present is very small an approximate result is obtained on the assumption that commercial glucose polarises  $+175^\circ$  V.

To estimate the approximate amount of commercial glucose used as adulterant in molasses, syrups, honey and similar preparations, Leach uses the formula  $g = (a - s)100/175$  where  $g = \%$  of commercial glucose,  $a =$  direct polarisation,  $s = \%$  of sucrose (*J. Amer. Chem. Soc.*, 1903, 25, 982).

In substances which consist largely of invert sugar much more accurate results are attained by polarising at  $87^\circ$  in a water-jacketed

tube an inverted half-normal solution of the sample (13 grm.) prepared as described elsewhere with the following exceptions: after inversion, cool, add a few drops of phenolphthalein and enough sodium hydroxide to neutralise; discharge the pink with a few drops of dilute hydrochloric acid, add from 5 to 10 c.c. of alumina cream, and make up to the mark and filter. Multiply by 2 the reading at 87° in the 200 mm. tube; multiply this result by 100 and divide by the factor 163 to express the glucose in terms of glucose polarising 175° V.

## HONEY

Ordinary honey is a saccharine substance collected and stored by a particular species of bee (*Apis mellifica*).

Honey is essentially a concentrated aqueous solution of certain sugars, dextrose and lævulose being the chief constituents. In some cases small amounts of sucrose are present and also a sensible quantity of the alcohol mannitol. Honeys, particularly when of coniferous origin, also sometimes contain some quantity of a carbohydrate intermediate in properties between starch and sugar, which is precipitated by strong alcohol. These are termed *honeydew* honeys in America. The other constituents are water, formic and other organic acids and small proportions of mineral and flavouring matters, wax and debris in the form of pollen, insects' wings, etc. Not infrequently alkaloidal and bitter principles derived from the pollen are also met with.

Genuine honey should contain not more than 8% of sucrose, 25% of water and 0.25% of ash. It should contain dextrose and lævulose in about equal proportions and be lævorotatory. Honey of coniferous origin, however, gives genuine dextrorotatory samples.

Although the figures representing the other constituents show considerable range, the great majority of samples of honey are of a remarkably constant character, the reducing sugars ranging from 70 to 80%, the water from 17 to 20%, and the ash from 0.10 to 0.25%. In normal honey the dextrose and lævulose are present in approximately equal proportions, but if the honey has crystallised in the comb the runnings therefrom will be deficient in dextrose, and hence will be strongly lævorotatory. It is held by experienced bee-keepers that all genuine honey will eventually crystallise, and hence that honey warranted to remain syrupy is probably adulterated.

The composition and analysis of American honeys has been very fully studied by C. A. Browne (*U. S. Dept. Agric., Bureau of Chemistry, Bulletin 110*, March, 1908), who has investigated the general composition of honeys with particular reference to the effects of different floral nectars. He gives the following average analyses of 99 samples. The table also includes the average of recent analyses of European honeys by König (*Chem. Nahrungs-und Genussmittel*, 3d edition)—138 samples—and by Lehman and Stadlinger—17 samples (*Zeitsch. Nahr.-Genussm.*, 1907, 13, 397).

	Browne			König			Lehman and Stadlinger
	Lævorotatory honeys, 92 samples	Dextro-rotatory honeys, 7 samples	American honeys, average, 99 samples	Minimum	Maximum	Average	
Water...	17.70	16.1	17.6	10.00	33.60	20.60	19.30
Invert sugar...	75.0	67.0	74.4	54.00	91.58	73.10	73.45
Sucrose	1.9	3.0	2.0		12.90	1.76	3.10
Ash...	0.18	0.81	0.23	0.02	0.68	0.25	0.09
Undetermined	3.70	3.40	3.70				4.07
Free acid as formic	0.08	0.12	0.09				0.07

The following figures give the average composition of genuine (Canadian?) honey (Canadian Dept. In. Rev., *Bull.* 47):

Sucrose (by Clerget),	0.5 to 7.64%
Dextrose and lævulose,	66.37 to 78.80%
Water,	12.0 to 33.00%
Ash,	0.03 to 0.50%

**Analysis of Commercial Honey.**—The common adulterants of honey are starch-sugar, invert sugar, sucrose, and molasses.

The proportion of *water* in honey may be estimated as in molasses (page 446), or by the method of Wiley, described on page 442. A useful check on the result is obtained by calculating the solids from the density of a 20% solution of the sample, as described on page 368.

The **ash** of genuine honey is usually very trifling in amount. If in excess of 0.3%, it should be tested for *calcium sulphate*, the presence of which, in notable quantity, is an almost certain indication of adulteration by starch glucose or invert sugar. Sulphates may also be detected by the direct addition of barium chloride to the aqueous solution of the sample. A high ash containing a notable proportion of *chlorides* points to a probable adulteration with molasses.

**The insoluble matter** of honey may be ascertained as in sugar. It usually consists of wax, pollen and some minor organised materials, and should be carefully examined under the microscope. **Starch**, which is not a normal constituent of honey, will be readily recognised in the residue by its reaction with iodine, and, if present in quantity, points to an adulteration of the sample with flour or other farinaceous substance, the exact nature of which will be indicated by its microscopic appearance.

**Gelatin**, if present, will be left undissolved on treating the sample with alcohol, and will be recognised by its odour on ignition, and the reaction of its aqueous solution with tannin.

**Dextrin**, which is not found in genuine honey, but is a constituent of commercial starch-sugar, may be detected by diluting the honey with an equal volume of water, and gradually adding strong alcohol, stirring constantly until a permanent turbidity is produced. In samples adulterated with starch-sugar a heavy gummy deposit will soon form, but with genuine honey only a slight milkiness is produced.

**Saccharine additions** to honey can only be detected by a careful examination of the action of the sample on polarised light, and its behaviour with Fehling's and other reducible solutions. The following table shows the specific rotatory power and cupric reducing power of mixtures of sucrose and invert sugar, containing 82% of the solid and 18% of water, and of average starch-sugar syrup, as compared with genuine honey. The table also shows the changes produced in solutions of the above saccharine matters by the action of invertase (page 383), by prolonged heating with dilute acid (page 360), and by fermentation with yeast (page 428):

	Cane sugar 82 % water 18 %	Invert sugar 82 % water 18 %	Average starch-sugar, syrup	Genuine honey
<i>Specific Rotatory Power for Sodium Ray:</i>				
Original substance.	+54.5	-18.9 at 15°	+92 to 100	+2 to -3
After treatment with invertase.	-19.9 at 15°	-18.9 at 15°	little altered	little altered
After prolonged heating with dilute acid.	-19.9 at 15°	-18.9 at 15°	+45	little altered
After fermentation with yeast	inactive	inactive	very notably dextrorotatory	0 to +4
<i>Cupric Reducing Power:</i>				
Original substance.	0.0	82	53	61 to 82
After treatment with invertase.	86.3	82	little altered	little altered
After prolonged heating with dilute acid.	86.3	82	82	little altered
After fermentation with yeast	0.0	0	very notable	0 to 2

**Invert Sugar.**—According to the table, there is a sensible difference between the rotation of *invert sugar* and genuine honey, but unfortunately this distinction does not always hold good, for if the honey has crystallised in the comb some of the dextrose is apt to remain there, and the honey drained therefrom will contain excess of lævulose, and be more strongly lævorotatory than is indicated by the figures in the table. Unless, therefore, the ash be excessive, or happen to contain calcium sulphate, the positive recognition of added invert sugar by such means is impossible.

The artificial honey made by Herzfeld's method (*Zeitsch. Ver. deut. Zuckerind.*, 1881, 31, 1988), which consists in heating 1000 grm. of refined sugar with 300 c.c. of water and 1.1 grm. of tartaric acid to boiling for 30 to 45 minutes, has a rich golden-yellow colour and a mild, pleasant flavour. On analysis, but for a deficiency in ash, it gives values agreeing very closely with those recorded for pure honeys.

To detect the presence of added invert sugar in honey it is necessary to have recourse to colorimetric tests. C. A. Browne uses aniline acetate, which gives a red or pink tint with furfuraldehyde produced at the high temperature of inversion by the Herzfeld and other processes, but no coloration with pure honey. The reagent is freshly prepared each time before use by shaking 5 c.c. aniline with 5 c.c. water and enough glacial acetic acid (2 c.c.) just to clear the emulsion. 1 to 2 c.c. of the reagent are allowed to flow down the walls of a test-tube on 5 c.c. of a solution of the honey in an equal weight of water. If, after gentle shaking, a red ring forms below the aniline layer and gradually spreads to the whole solution, invert sugar is present.

Fiehe (*Chem. Zeit.*, 1900, 32, 1045) describes the following method: The ethereal extract of artificial honeys on evaporation gives an intense red coloration with resorcinol hydrochloric acid (1 part of resorcinol and 100 parts of hydrochloric acid, sp. gr. 1.19). This is due to decomposition products of lævulose formed by heating invert sugar with acids during the manufacture. Natural honey which has not been heated with acid never gives the reaction, nor does dextrose, galactose, lactose or maltose. Fiehe's reaction has been the subject of much further investigation. According to Quantin (*Ann. Chim. Anal.*, 1910, 15, 299) the reaction is valueless if the honey has been heated, but Witte (*Zeitsch. Nahr.-Genussm.*, 1911,

21, 305) states that honey that has only been heated in the usual way during its preparation occasionally gives a faint coloration with Fiehe's reagent, but this cannot be mistaken for the cherry-red colour given by artificial honey. According to Voerman and Bakker (*Zeitsch. öffentlich. Chem.*, 1911, 24, 461) genuine honey, heated for 6 hours in a boiling water-bath (whereby it is rendered decidedly unpalatable), or for 3 hours at  $105^{\circ}$ , only gives a transient and very slight rose colour with Fiehe's reagent. Hence, any heating of a genuine honey that can lead to a positive reaction with Fiehe's test must be very excessive, and it is considered that the reaction, in conjunction with other tests, is a very valuable aid in detecting added invert sugar.

The ammoniacal silver test of Ley (*Pharm. Zeitsch.*, 1902, 47, 603) consists in heating a solution of the honey with a small quantity of silver oxide dissolved in ammonia. Clear honeys give a brownish colour and leave a yellowish-green on the surface of the glass. Honey substitutes appear a dirty brown or black and give no greenish after-hue. According to Quantin (*Ann. Chim. Anal.*, 1910, 15, 299) Ley's test is valueless if the honey has been heated.

**Sucrose.**—Any considerable proportion of *sucrose* in honey would be indicated by the strong dextrorotation of the sample, changed to left-handed rotation on treatment with invertase or dilute acid. The proportion of sucrose can be estimated from the extent of the *change* in the rotatory and reducing power of the sample caused by treatment with invertase, or, in the absence of starch-sugar, by inversion with dilute hydrochloric acid, as on page 360. As already stated, a small percentage of sucrose appears sometimes as a constituent of genuine honey. For calculating the percentage of sucrose, Lehmann and Stadlinger (*Zeitsch. Nahr. Genussm.*, 1907, 13, 397) give the formula:

Per cent. sucrose =  $[\alpha]_D \times 1.1448$ , in which  $[\alpha]_D$  is the difference in the specific rotatory power before and after inversion.

**Starch-sugar** is still more dextrorotatory than sucrose to commence with, the optical activity falling to about one-half by prolonged treatment with acid, whilst the products left after fermentation are still notably dextrorotatory.

E. Beckmann (*Zeitsch. anal. Chem.*, 1896, 35, 263) tests honey for the addition of starch-sugar by means of methyl alcohol, which produces no precipitate with genuine honeys, including both the ordinary

form and the dextrorotatory variety. When starch-sugar is present there is a marked precipitate which should give the characteristic red coloration of erythrodextrin with iodine. The test has been extended so as to apply also to solid starch-sugar as follows: 5 c.c. of a 20% solution of honey in water are mixed with 3 c.c. of 2% barium hydroxide solution and 17 c.c. of methyl alcohol and the mixture is well shaken. Pure honey remains clear, but the above adulterants cause a considerable precipitate. Methyl alcohol of high purity must be used.

This method has been applied quantitatively, but is of doubtful accuracy in such cases. However, it does enable the analyst, even under unfavourable conditions, to recognise the addition of small quantities of dextrin, starch-sugar or its syrup to conifer honey containing as much as 4% of natural dextrinous matter. (This subject is dealt with in more detail in Leffmann and Beam's *Food Analysis*.)

Fiehe (*Pharm. Zentralbl.*, 1909, 50, 900) proposes the following method for detecting starch-sugar in honey: 10 grm. of the honey are warmed with 20 grm. of water on the water-bath and treated with 1 c.c. of 5% tannin solution and allowed to stand for some hours, the precipitated albuminoid matter then being filtered off. 2 c.c. of the filtrate are treated with 2 drops of concentrated hydrochloric acid and 20 c.c. of 94% alcohol. In the case of pure honey the mixture remains perfectly clear, but in the presence of starch-sugar a milky turbidity will be seen.

According to Leffmann and Beam, a common method of adulteration consists in pouring starch-sugar syrup over honey-comb from which the honey has been already drained. On standing, such a mixture acquires a honey flavour; it will give a high dextrorotatory polarisation hardly altered on hydrolysis.

The dextrin-like body in coniferous honey has been shown by König and Hörmann (*Zeitsch. Nahr.-Genussm.*, 1907, 13, 113) to differ from dextrans prepared from starch by malt extracts or acids. It is fermented by beer yeasts.

The reliable points in the differentiation of honey-dew honeys and those adulterated with glucose are (a) the difference in invert polarisation between 20° and 87° (corrected to 77% of invert sugar)<sup>1</sup>; (b)

<sup>1</sup> To do this the difference in invert polarisation between 20° and 87° is multiplied by 77, the average % of invert sugar in pure honey, and this product divided by the % of invert sugar after inversion found in the sample. To find the % of pure honey in the sample this quotient is multiplied by 100 and divided by 26.7.

the reaction of the honey and its precipitated dextrin toward iodine; (c) the polarisation of the inverted solution after precipitation of the dextrin with absolute alcohol. This process due to König and Karsch (*Zeitsch. anal. Chem.*, 1895, 34, 1) depends on the fact that after precipitation of the dextrans and inversion natural honeys will show a lævorotation, honeys with 25% or more of glucose a dextrorotation.

**Diastase Test.**—Auzinger (*Zeitsch. Nahr.-Genussm.*, 1910, 19, 65) has shown that the enzymes of natural honey afford a useful aid in discriminating between the latter and artificial honey. The test for diastase may be made as follows: A mixture of the honey and 1% starch paste is kept at about 45° for 1 hour and then tested with iodine solution; if diastase be absent the intense blue colour characteristic of starch is produced, whilst in presence of diastase the starch is hydrolysed and the colour of the solution, after addition of iodine, will be yellow. It is advisable to use various proportions of honey and starch paste and in this way the diastatic activity of the honey can be determined. According to Gothe (*Zeitsch. Nahr. Genussm.*, 1914, 28, 286), the most aromatic honeys possess the highest diastatic values, honeys from sugar-fed bees have low diastatic values, whilst artificial and over-heated honeys have no, or only very slight, diastatic activity. When undiluted honey is heated for 1 hour at 65°, a slight decrease in diastatic activity is observed; at 85° to 90° it is destroyed.

**Albumin Test.**—To distinguish between genuine and artificial honey Lund (*Mitt. Lebensmittel. Hyg.*, 1911, 1, 38) determines the albumin with a sulphuric acid solution of phosphotungstic acid. 20 c.c. of a filtered 10% solution of the honey are treated with 5 c.c. of the reagent (2 grm. phosphotungstic acid dissolved in 20 grm. sulphuric acid, 1 : 4, and 80 c.c. of water) in a graduated tube. The mixture is diluted with 20 c.c. of water, mixed and left for 24 hours, the volume of the precipitate then being read; this varies from 0.6 to 2.7 c.c. for natural honeys and from 0 to 0.5 c.c. for artificial honeys.

## MAPLE PRODUCTS

**Maple syrup<sup>1</sup> and maple sugar** are products of considerable importance in the United States, but have not yet come to England

<sup>1</sup>Maple Syrup is defined to contain not more than 32% of water and not less than 0.45% of ash.

in any quantity. They contain sucrose with minute amounts of special flavours and are frequently adulterated with sucrose from other sources or starch-sugar. The latter is detected by polarimetric examination before and after hydrolysis when pure maple sugar is inverted and has a negative rotatory power, whereas glucose is but slightly affected.

Leffmann and Beam quote the following results obtained by Ogden:

	Polarimeter reading		% sucrose
	Direct	After hydrolysis	
Maple syrups free from glucoses,	53.1 59.6	-22.2 -21.9	56.0 60.6
Maple sugars,	84.1 88.0	-28.8 -28.3	85.9 87.6
Maple syrups containing glucoses,	80.0 100.0	+18.9 +45.6	

The following abridged table (*U. S. Dept. Agric., Bulletin No. 466, Nov. 3, 1917*), based on the analysis of 283 samples of maple sugar manufactured in the United States and of 80 samples of Canadian maple sugar, shows the limits within which the constituents are liable to vary.

	Sucrose, %	Invert sugar, %	Ash, %	Sol. ash Insol. ash	Alkalinity sol. ash Insol. ash	Lead number (Winton)	Malic acid value
<i>U. S. A.:</i>							
Maximum	98.62	37.30	1.66	4.97	2.29	4.95	1.72
Minimum	57.94	0.09	0.76	0.61	0.37	1.85	0.51
Mean	91.89	5.46	0.95	1.88	0.90	2.68	0.91
<i>Canada:</i>							
Maximum	96.59	35.26	1.70	3.20	1.54	4.11	1.51
Minimum	58.92	0.88	0.76	0.43	0.40	1.86	0.62
Mean	86.48	8.76	1.06	1.36	0.76	3.01	1.03

Pure maple syrup gives an abundant flocculent precipitate with methyl alcohol which does not adhere to the glass. When much starch-sugar is present a more granular precipitate is obtained which adheres to the glass.

Leach (*Bulletin* 65) finds that the grade of starch-sugar syrup commonly used to adulterate maple syrup, honey, molasses, etc., has a value  $[a]_D = 87.5$  with a half-normal weight in a 2 dm. tube. He calculates approximately the amount present ( $G$ ) from the formula  $G = 0.561 (a - S)$ , in which  $S$  = sucrose and  $a$  the polarimeter reading before hydrolysis. The sucrose is determined in the usual manner by the change in rotation on hydrolysis.

To judge the addition of sucrose the amount and alkalinity of the ash are carefully determined, also the amount of basic lead acetate precipitate and the malic acid value. The ash should not be less than 0.625% of the total sucrose; it should be estimated by burning in a muffle at a low temperature as some of the constituents are volatile; it is also deliquescent and must be weighed quickly. The alkalinity of the water-soluble portion to phenolphthalein and methyl orange and of the insoluble portion can be estimated in the usual manner.

Hortvet (*J. Amer. Chem. Soc.*, 1904, **26**, 1523) measures the volume of the lead precipitate after concentration by a centrifuge. Adulterated articles give much less precipitate.

Winton and Kreider (*Ibid.*, 1906, **28**, 1204), instead of measuring the volume of the precipitate produced by adding basic lead acetate to maple products, estimate the amount of lead in this precipitate. The lead numbers vary from 1.2 to 1.77 for genuine syrups and 1.8 to 2.5 for sugars.

The tentative A. O. A. C. method for estimating the lead number (Winton, *J. Amer. Chem. Soc.*, 1906, **28**, 1204) is as follows:

### Reagents

*Standard Basic Lead Acetate Solution.*—Boil 430 grm. of neutral lead acetate and 130 grm. of litharge for 30 minutes, or boil 560 grm. of Horne's dry basic lead acetate with 1 litre of water, cool, allow the mixture to settle, and dilute the supernatant liquid to 1.25 sp. gr. To a measured amount of this solution add 4 volumes of water and filter if not perfectly clear. The solution should be standardised each time a set of determinations is made. If the directions for preparing the basic lead acetate are not carried out carefully, the use of Horne's dry basic lead acetate is preferable.

*Determination of Lead in the Blank.*—Transfer 25 c.c. of the standard basic lead acetate solution to a 100 c.c. flask, add a few

drops of acetic acid, and make up to the mark with water. Shake and determine lead sulphate in 10 c.c. of the solution as directed below. The use of the acid is imperative in this case to keep the lead in solution when diluted with water.

**Estimation.**—Transfer 25 grm. of the sample to a 100 c.c. flask by means of water. Add 25 c.c. of the standard basic lead acetate solution and shake, fill to the mark, shake and allow the mixture to stand for at least 3 hours before filtering. Pipette 10 c.c. of the clear filtrate into a 250 c.c. beaker, add 40 c.c. of water and 1 c.c. of concentrated sulphuric acid, shake and add 100 c.c. of 95% alcohol. Allow the mixture to stand over night, filter on a weighed Gooch crucible, wash with 95% alcohol, dry in a water-oven and ignite in a muffle or over a Bunsen burner, applying the heat gradually at first and avoiding a reducing flame. Cool and weigh. Subtract the weight of lead sulphate so found from the weight of lead sulphate found in the blank and multiply by the factor 27.33. The use of this factor gives the lead number directly without the various calculations otherwise required.

The tentative A. O. A. C. method for estimating the malic acid value is as follows:

Weigh 6.7 grm. of the sample into a 200 c.c. beaker, add 5 c.c. of water, then 2 c.c. of a 10% calcium acetate solution and stir. Add gradually, and with constant stirring, 100 c.c. of 95% alcohol and agitate the solution until the precipitate settles, or allow the mixture to stand until the supernatant liquid is clear. Filter off the precipitate and wash with 75 c.c. of 85% alcohol. Dry the filter paper and ignite in a platinum dish. Add 10 c.c. of N/10 hydrochloric acid and warm gently until all the lime dissolves. Cool and titrate back with N/10 sodium hydroxide using methyl orange as an indicator. The difference in c.c. divided by 10, represents the malic acid value of the sample. Previous to use the reagents should be tested by a blank determination and any necessary corrections applied.

The following tests, due to Sy (*J. Amer. Chem. Soc.*, 1908, 30, 1429), are stated to be of considerable value.

**Colour Test.**—15 c.c. of syrup or 15 grm. of sugar and enough water to make 15 c.c. are very thoroughly mixed in a test-tube with 3 c.c. of pure amyl alcohol and 1 c.c. of a 20% solution of phosphoric acid and allowed to stand until the alcohol separates. The alcoholic layer shows a decided brown colour with pure maple products; with

adulterated samples the colour varies from a very pale to a light brown, according to the proportion of maple product; cane products containing caramel give no colour.

*Foam Test.*—5 c.c. of syrup are treated in a narrow tube graduated to 0.1 c.c. with 10 c.c. of water, the mixture is thoroughly shaken for half a minute and allowed to stand for 10 minutes, when the volume of foam is read. With pure maple products the amount is never less than 3 c.c., whereas adulterated products all give less.

*Volume of Basic Lead Acetate Precipitate.*—5 c.c. of syrup or 5 gm. of sugar and water to make 5 c.c. are placed in a glass-stoppered 25 c.c. measuring cylinder with 10 c.c. of water and 2 c.c. of basic lead acetate solution. The whole is thoroughly mixed and allowed to settle for 20 hours when the volume of the precipitate is read. For pure maple products this will be over 3 c.c. and is usually over 5 c.c. With adulterated products the volume is less.

## GLUCOSIDES

The name glucoside is applied to a numerous class of substances occurring in plants and seeds which yield a glucose,  $C_6H_{12}O_6$ , generally dextrose, on hydrolysis with acids. The other constituents yielded by glucosides are numerous, comprising alcohols, phenols, most of the vegetable dye-stuffs, mustard oil, many poisons, etc. A class of glucosides of some industrial importance, since they are poisonous and yield hydrogen cyanide on hydrolysis, are known as cyanogenetic glucosides. They have been found in many fodder plants. Most of the glucosides are accompanied in the plant by an enzyme capable of hydrolyzing them. Enzyme and glucoside are stored in different cells in the plant, but act on one another when the plant is macerated with water. To extract a glucoside, therefore, it is first necessary to destroy the enzyme; this is often effected by boiling the plant with alcohol. Whilst the action of the enzyme is very specific and limited, as a rule, to a few closely related compounds, very many of the glucosides which are  $\beta$ -dextrose derivatives are hydrolysed by emulsin, an enzyme very widely distributed in plants and conveniently prepared from almonds.

The detection and determination of a glucoside in plant products is often best performed on the non-sugar component. Provided sucrose or other di-saccharides are not present, the change in cupric

reducing power on hydrolysis by acids may be made use of. It is often preferable to extract the glucosides by means of solvents and weigh as such.

Bourquelot (*Arch. Pharm.*, 1907, **245**, 172) has elaborated a method for their detection by means of emulsin which he prepares by crushing blanched almonds in a mortar and macerating each 100 grm. with 200 c.c. of distilled water containing chloroform for 24 hours at the normal temperature. The mixture is strained, pressed, the protein precipitated by 10 drops of glacial acetic acid and, after filtering, the enzyme precipitated by 95% alcohol. The enzyme is collected, washed with a mixture of equal volumes of ether and alcohol, and dried *in vacuo*. It is obtained as a white powder.

To detect glucosides, the material is first boiled with 95% alcohol, any acidity neutralised with calcium carbonate, the alcohol is distilled off and the residue made up to 250 c.c. with water containing a little thymol. Since the emulsin sometimes contains invertase, the material is first treated with invertase to eliminate sucrose, as described on page 383, heated to boiling for a few minutes to destroy the invertase and then treated with the emulsin preparation. The optical rotation is ascertained before and after hydrolysis; a change, if observed, denotes the presence of a  $\beta$ -glucoside of dextrose.

The problem often presents itself which of the glucose sugars is present in a glucoside. Ter Meulen (*Rec. Trav. Chim.*, 1905, **24**, 444) applies the principle, independently discovered by E. F. Armstrong (*Proc. Roy. Soc.*, 1904, **73**, 516), that the rate of action of a particular enzyme is hindered only by that sugar the glucosidic derivative of which is hydrolysed by the enzyme.

By measuring the rate of hydrolysis of the glucoside by its enzyme in the presence of various other sugars one only is found to retard, and this is the sugar present in the glucoside.

To ascertain whether a glucoside is a derivative of  $\alpha$ - or  $\beta$ -dextrose, E. F. Armstrong hydrolyses with an active enzyme for half an hour and determines the change in optical rotatory power produced by the addition of a drop of aqueous ammonia. A decrease denotes the presence of  $\alpha$ -dextrose, an increase that of  $\beta$ -dextrose. Dunstan, Henry and Auld (*Proc. Roy. Soc.*, 1907, **B 79**, 315, 322) have applied this method with success to the identification of  $\alpha$ -dextrose in phaseolunatin.

Cyanogenetic glucosides are best determined by means of the hydrogen cyanide they produce when hydrolysed, as it is the poisonous nature of the feeding stuffs in which they occur which is really in question. Henry and Auld (*J. Soc. Chem. Ind.*, 1908, 27, 428) have described the following method of procedure which gives the maximum amount of hydrocyanic acid obtainable. The product is ground as rapidly as possible, weighed, placed in a Soxhlet extraction apparatus and reprecipitated with hot alcohol so as to dissolve out the glucoside. The solvent is distilled off and the residue mixed with 50 c.c. of water and 10 c.c. of 10% hydrochloric or sulphuric acid added. The mixture is then distilled, preferably in a current of steam, until hydrocyanic acid can no longer be found in the distillate in which it may be estimated volumetrically by Liebig's method. The authors prefer to add a slight excess of sodium hydrogen carbonate and titrate with an excess of iodine solution. A little of the freshly ground product is macerated with water in presence of an antiseptic to ascertain whether hydrogen cyanide is formed, thereby denoting the presence of the enzyme.

### URINE ANALYSIS

Sugars in urine may be detected and estimated either polarimetrically or by means of their reducing power or by the other usual methods elsewhere described. Normal urine contains, at the most, only traces of dextrose, but on the other hand, traces of substances other than reducing sugars, such as creatinine and uric acid, also glucuronic acid are normally present, all of which reduce Fehling's solution. Moreover, other optically active substances are generally present, so that urine requires a preliminary treatment before applying the sugar tests, and it is necessary to make confirmatory tests to be sure that sugar is present. Too great a stress must not be laid on the presence of an insignificant proportion of sugar. It is desirable before applying the sugar tests to remove proteins, if present, by adding a few drops of acetic acid, heating to boiling and filtering from any precipitate formed.

The liquid should then be rendered distinctly alkaline with sodium hydroxide, filtered from any precipitate, and the copper solution employed in the following manner:

Heat to boiling in a test-tube 10 c.c. of Fehling's solution, prepared in the usual way, a few small fragments of clay pipe-stem being

previously introduced to prevent bumping. When boiling, add 0.5 to 1 c.c. of the urine previously treated as indicated above. If sugar is abundant, a yellowish or brick-red opacity and deposit will be produced. If a negative reaction is obtained, test for traces of sugar by adding 7 c.c. or 8 c.c. of the urine to the hot liquid, heating again to boiling, and then setting the tube aside for some time. If no turbidity is produced as the mixture cools, the urine is either quite free from sugar or at any rate contains less than 0.025%. If the quantity of sugar present is small—that is, under 0.5%—the precipitation of the yellow or red cuprous oxide does not take place immediately, but occurs as the liquid cools, the appearance being somewhat peculiar. The liquid first loses its transparency, and passes from a clear bluish-green to an opaque, light-greenish colour. This green milky appearance is quite characteristic of dextrose.

The colours of the precipitates obtained are attributed by some authors to the proportion of alkali present, the yellow and green precipitates being forms of cuprous hydroxide. In other cases the disturbance is said to be due to the presence of creatinine. On adding Fehling's solution to a solution of this substance, a green liquid is produced, and on boiling, a yellow colouration is observed, without, however, any separation of cuprous oxide. It is this behaviour which causes interference with the detection of reducing sugars, the combination of the yellow and blue colours resulting in a green; in addition, the creatinine compound is said to have the power of preventing the precipitation of cuprous oxide by reducing sugars.

Nylander's test, which is not affected by creatinine or uric acid, consists in boiling the urine for 2 to 5 minutes with a small quantity of a solution containing 100 grm. of sodium hydroxide (sp. gr. 1.12), 4 grm. of potassium sodium tartrate and 2 grm. of bismuth subnitrate, when a black precipitate forms on cooling.

The detection and estimation of sugars in urine offers but little difficulty when the amount is 0.25% or over, but when the quantity is very small satisfactory results are not often attainable. The occurrence of sugar normally in the urine has been much disputed. By the use of phenylhydrazine—a method free from the objections and fallacies which underlie nearly all other tests—it seems proved that, while normal human urine may sometimes contain traces of sugar, that substance is by no means constantly present, and a great number of the recorded observations are quite inconclusive.

It is important to consider the extent to which non-sugar substances interfere, and the manner in which they may be removed or their influence obviated. The chief of these are uric acid, xanthine, and creatinine, but under some conditions urine contains glucuronic acid or compounds thereof which simulate sugar very closely. The amount of uric acid passed per diem under ordinary conditions is said to be about 0.5 gm., though, of course, in many instances it is considerably more. Xanthine and the allied bodies are present in still smaller amount. According to Voit, the proportion of creatinine passed in 24 hours ranges from 0.5 to nearly 5 gm. Urine containing the latter amount would exert a reducing action on Fehling's or Pavy's solution equivalent to the presence of 0.32% of dextrose.

Dextrose in urine may be estimated by:

1. Titration with Fehling's solution in the usual manner.
2. Titration with Pavy's solution.
3. Titration with Knapp's mercurial solution.
4. Polarisation.
5. Fermentation.

For ordinary clinical purposes very great accuracy is not required. In such cases as the proof of a diminution in the amount of sugar following treatment, the errors of collecting urine properly and multiplication so as to give the daily output more than counterbalance slight errors in the estimation.

Before polarising, the urine may be clarified and freed from proteins, uric acid, phosphates and colouring matters by treatment with alumina cream or with basic lead acetate. Thus, 100 c.c. of urine of known sp. gr. are measured into a flask, 5 c.c. of the clarifying reagent added, the solution made up to 110 c.c., shaken, filtered and polarised. To make certain that the rotation obtained is really due to dextrose, the urine is examined polarimetrically before and after fermentation, the change in rotatory power indicating the amount of dextrose present.

The fermentation test for dextrose in urine is very useful for confirmatory purposes, and also serves to distinguish between dextrose and unfermentable pentoses, lactose or glucuronic acid. Fermentation at 34° to 36° should be complete in 6 hours. If the operation be

prolonged, the gas formed is probably due to other changes. The Lohnstein saccharometer is often used in physiological laboratories for this purpose.

Fehling's solution is used either gravimetrically or volumetrically in the ordinary manner.

*Modified Fehling's Solution.*—For examination of urine the following modification of the copper solution is strongly recommended by S. R. Benedict (*J. Biol. Chem.*, 1909, 5, 485).

Copper sulphate (cryst.), 8.65 grm.  
Sodium citrate, 86.50 grm.  
Sodium carbonate (dry), 50.00 grm.

The sodium citrate and carbonate are dissolved in 300 c.c. of water, filtered if necessary, and made up to 425 c.c. The copper sulphate is dissolved in 50 c.c. of water and made up to 75 c.c. The solutions are mixed. The mixture keeps well. Benedict found that commercial sodium citrate is satisfactory. The solution is not reduced by uric acid, chloroform, chloral, or formaldehyde.

Pavy's solution may also be used for the estimation of the glucose in diabetic urine, though it cannot be employed for the detection of small quantities of the sugar. Müller and Hagen estimate the sugar volumetrically by Knapp's mercurial solution, which has the advantage of being applicable to samples of urine containing as little as 0.1% of glucoses, whilst Fehling's solution cannot be applied quantitatively in the ordinary manner if less than 0.5% of dextrose be present, owing to the incomplete separation of the cuprous oxide in presence of certain obscure foreign matters contained in urine.<sup>1</sup>

To render urine fit for the application of Fehling's solution, Carne-lutti and Valente recommend that 100 c.c. of the sample should be evaporated to a syrup on the water-bath, 1 c.c. of a 25% solution of zinc chloride previously mixed with one-fourth of its volume of hydrochloric acid is added, then 2 volumes of absolute alcohol, and the whole allowed to stand for some hours. The liquid is then filtered, the residue washed with alcohol, the alcohol evaporated from the solution and the residual liquid made up to 100 c.c.

<sup>1</sup> J. G. Otto recommends that, for titrating solutions containing 1 to 0.5% sugar, the Knapp's solution should be diluted with 4 volumes of water, for those containing 0.5 to 0.1%, with 3 volumes of water, whilst for solutions containing less than 0.1%, 2 volumes of water should be added. In all cases the urine should be added gradually to the mercurial solution.

with distilled water. In this solution excellent results are said to be obtainable by Fehling's solution.

Copper sulphate yields at first little or no precipitate with normal urine in the cold but, on standing or boiling, a pale green precipitate is thrown down which has a tendency to darken if the heating be continued. If copper acetate be used, or sodium acetate with copper sulphate, the precipitation is more complete, uric acid, xanthine, hypoxanthine, colouring matter, and albumin being entirely thrown down, and creatinine and phosphates partially precipitated. The filtered liquid cannot be used for the phenylhydrazine test, and the presence of copper unfits it for titration with Pavy's solution; but it is admirably suited for the detection of small quantities of sugar by Fehling's solution, as follows:

From 7 to 8 c.c. of the sample are heated to boiling, and, without separation of any precipitate of proteins, 5 c.c. of the solution of copper sulphate used for preparing Fehling's solution are added, and the liquid again boiled. This produces a precipitate, principally uric acid, xanthine, hypoxanthine, and phosphates. To render the precipitation complete, however, it is desirable to add to the liquid, when partly cooled, from 1 to 2 c.c. of a saturated solution of sodium acetate having a feebly acid reaction. The liquid is filtered, and to the filtrate, which will have a bluish-green colour, 5 c.c. of the alkaline tartrate mixture used for preparing Fehling's solution are added, and the liquid boiled for 15 to 20 seconds. In the presence of more than 0.25% of sugar, separation of cuprous oxide occurs before the boiling point is reached, but with smaller proportions precipitation takes place during the cooling of the solution, which becomes greenish and opaque, and suddenly deposits cuprous oxide as a fine orange-yellow precipitate. When a urine rich in sugar is under examination, the volume taken can be advantageously reduced from 7 or 8 c.c. to 2 or 3 c.c., or even less, water being added to replace it.

It is evident that in this modification of the ordinary Fehling's test advantage is taken of the very general precipitating power of cupric acetate to remove from the urine the great majority of those substances which interfere with the detection of sugar, by themselves reducing the alkaline copper solution, retaining the cuprous oxide in solution, or producing a flocculent precipitate which masks the true reaction of sugar. Operating as described above, no greenish

turbidity refusing to settle is produced, and hence the separation of any cuprous oxide is very readily observed. It is important that the sodium acetate should not be added till the liquid has partly cooled, so as to avoid any chance of reaction of the resultant cupric acetate with the glucose in the manner observed by Barfoed (see page 426).

Pavy's method of estimating sugar by titration with ammoniacal cupric solution would probably be more generally applied if it did not necessitate the use of a special apparatus. The following form of the test is simple and convenient, but less accurate than where larger quantities of the urine and reagent are employed. An accurately measured volume of 10 c.c. of Pavy's solution is placed in a wide test-tube, a few fragments of tobacco-pipe dropped in, and 8 to 10 drops of petroleum or paraffin burning oil added. This forms an upper layer which effectually excludes the air. The test-tube is inserted into the neck of a wide-mouthed flask containing hot water, which is then heated until the contents of the tube have reached the boiling point. The urine to be tested is treated with an equal volume of ammonia and filtered from the precipitated phosphates. A known volume of the filtrate is then further diluted with a definite quantity of water, according to the proportion of sugar supposed to be present, and then added, drop by drop, to the boiling-hot Pavy's solution by means of a small burette or graduated pipette, until the disappearance of the blue colour indicates the termination of the reaction. If 10 c.c. of Pavy's solution were employed, the volume of urine required to decolorise it contains 0.005 grm. of sugar.

Unclarified healthy human urine may exert a reducing action on Pavy's solution equal to that of a liquid containing from 0.1 to 0.3% of dextrose. Of this, one-quarter is ascribed to uric acid (removable by lead acetate) and the remainder to creatinine (removable by mercuric chloride).

The phenylhydrazine test for dextrose has a special value, as it is not given by the other non-sugar reducing substances in the urine. To apply it, 50 c.c. of the suspected urine, previously freed from protein, are heated in the boiling water-bath for an hour with 10 to 20 drops of phenylhydrazine and the same volume of 50% acetic acid. 5 grm. of sodium chloride may be added to facilitate precipitation. If any quantity of dextrose is present, an orange-yellow, generally crystalline, precipitate separates in the hot liquid or on cooling. This should be filtered when cold, well washed with water

to remove excess of phenylhydrazine and crystallised from a small quantity of dilute alcohol, when characteristic yellow needles are obtained, melting at  $205^{\circ}$  and practically insoluble in boiling water.

When only minute traces of sugar are present, the complete separation of the glucosazone requires some time, but the qualitative indication is readily and quickly obtained. Dextrose and lævulose yield the same glucosazone; the pentoses and glucuronic acid (see later) also yield insoluble compounds with phenylhydrazine.

It is important that the phenylhydrazine used should be of good quality. It should be almost straw-yellow in colour and is conveniently kept in sealed bottles containing only a small quantity, which can be quickly used when once the bottle has been opened.

Salkowski takes 5 c.c. of urine, 0.5 c.c. of glacial acetic acid and 20 drops of phenylhydrazine, boils for 1 minute, adds 5 drops of 15% sodium hydroxide, and a volume of water equal to three-fourths of the original volume and heats nearly to boiling. After standing 24 hours, a sulphur-yellow precipitate of slender needles is obtained if dextrose is present, but not from lactose or maltose.

Unfortunately, the phenylhydrazine test cannot be applied quantitatively, though the amount of precipitate formed gives a fair indication of the proportion of sugar present.

**Glucuronic Acid.**—As already stated, glucuronic acid simulates the behaviour of dextrose very closely, and gives not only all the ordinary reactions as a reducing agent, but is the only other constituent of urine which reacts with phenylhydrazine.

Glucuronic acid is a syrupy liquid, miscible with alcohol or water. When the aqueous solution is boiled, evaporated, or even allowed to stand at the ordinary temperature, the acid loses the elements of water and yields the anhydride or lactone ( $C_6H_8O_6$ ), which forms monoclinic tables or needles, having a sweet taste and melting at  $167^{\circ}$ . The lactone is insoluble in alcohol, but is dissolved by water to form a solution which is dextrorotatory ( $[\alpha]_D = 19.25^{\circ}$ ), prevents the precipitation of cupric solutions by alkalis, and vigorously reduces hot Fehling's solution, the cupric reducing power being 98.8 compared with dextrose as 100. The acid is dextrorotatory ( $[\alpha]_D = 35^{\circ}$ ), but many of its compounds are lævorotatory. It reduces Fehling's solution on heating, and precipitates the metals from hot alkaline solutions of silver, mercury, and bismuth. With phenylhydrazine, glucuronic acid forms a yellow crystalline com-

pound, melting at  $114^{\circ}$  to  $115^{\circ}$ , and closely resembling phenylglucosazone. When oxidised with bromine, glucuronic acid yields saccharic acid, which can be again reduced to glucuronic acid by treatment with sodium amalgam. It is distinguished from dextrose by not undergoing alcoholic fermentation when treated with yeast.

It gives the orcinol and phloroglucinol reactions for pentoses, as it is dehydrated on boiling with hydrochloric acid, yielding furfural and carbon dioxide, but the production of furfural is much slower than in the case of the pentoses. The carbon dioxide evolved may be weighed to estimate glucuronic acid. To effect this, Lefevre and Tollens (*Ber.*, 1907, 40, 4513) boil with hydrochloric acid (sp. gr 1.06) for 3.5 hours and aspirate a current of pure air through the apparatus; the carbon dioxide is washed and absorbed in potash bulbs and weighed. The results are, as a rule, too high owing to the presence of other substances which yield carbon dioxide.

Combined with the estimation of the furfural, this method affords a simultaneous estimation of pentoses and glucuronic anhydride. Three parts of glucuronic anhydride give one part of furfural phloroglucide.

Tollens (*Ber.*, 1908, 41, 1788) finds that glucuronic acid alone, and not the pentoses, form a blue substance, on heating with naphthoresorcinol and hydrochloric acid, which is soluble in ether. This enables glucuronic acid to be identified with certainty in presence of pentoses.

**Other Sugars in Urine.**—Recent researches have shown the occasional presence of other sugars, besides dextrose, in pathological urine. The reducing action of a urine may indicate dextrose, lævulose, lactose, pentoses or glucuronic acid; the fermentation test only dextrose and lævulose.

These sugars are best detected by means of the substituted phenylhydrazines.

To detect pentoses in urine, the orcinol test is carried out as follows: 0.03 grm. of powdered orcinol is dissolved in 10 c.c. of fuming hydrochloric acid and a drop of dilute ferric chloride added. 5 c.c. of this solution and 2 c.c. of urine are placed in a tube closed with a plug of cotton wool and heated nearly to boiling. If pentoses are present an emerald-green coloration gradually appears, which soon becomes dark green.

To estimate pentoses in urine, Jolles (*Zeitsch. anal. Chem.*, 1907, 46, 764) proceeds as follows: The urine is boiled with a few drops of acetic acid and concentrated, if necessary, to free it from interfering volatile substances. 100 c.c. are distilled with 150 c.c. of hydrochloric acid (sp. gr. 1.06) in a current of steam until the distillate amounts to 1000 c.c. 100 c.c. of this are neutralised with an excess of 20% sodium hydroxide, methyl orange being used as indicator; N/2 hydrochloric acid is added to restore the red coloration, and the liquid titrated with sodium hydrogen sulphite and standard iodine solution.

For further information consult "*Analyse des Harns.*"

### PENTOSEs

The best known pentoses are arabinose and xylose. Even more important are their polymerides—the pentosans.

Arabinose and xylose, in the absence of other sugars, may be detected and estimated in the same manner as dextrose—either polarimetrically or by means of copper or mercuric solutions.

Characteristic are the colorations obtained with alcoholic solutions of phenols and hydrochloric or sulphuric acid, on cautious heating. Orcinol gives a bluish-violet coloration in the cold, and on warming, a reddish colour, changing to violet-blue, and finally bluish-green flakes separate, which dissolve in alcohol, yielding a solution having a characteristic absorption-spectrum. The orcinol reagent is prepared by dissolving 1 grm. of orcinol in 200 c.c. of 94% alcohol. 3 drops are added to 5 c.c. of the sugar solution and then 5 c.c. of concentrated hydrochloric acid. The mixture is shaken and heated on a boiling water-bath for half an hour. The method is not available in presence of lævulose, a bronze brown coloration being then obtained (Pieraerts, *Bull. Assoc. Chim. Sucr.*, 1908, 26, 46).

Phloroglucinol and hydrochloric acid produce a bright cherry-red coloration on heating and the solution so prepared has a very characteristic spectrum.

A more general method, which is applicable also to the pentosans, consists in distillation with hydrochloric acid in a current of steam, whereby furfural is formed and may then be estimated by means of its compounds with phloroglucinol, phenylhydrazine or sodium hydrogen sulphite, or by its reducing action on Fehling's solution.

Of these the phenylhydrazine method is least satisfactory. When using sodium hydrogen sulphite, an aliquot portion of the distillate is mixed with a known volume in excess of the standard hydrogen sulphite. After 2 hours' standing, this excess is estimated by titration with standard iodine solution: 1 c.c. of normal sodium hydrogen sulphite solution is equivalent to 0.07505 grm. of pentose.

The phloroglucinol method has been worked out in great detail by Kröber (*J. Land.*, 1900, **48**, 357; 1901, **49**, 7) and adopted as official by the A. O. A. C. The details are as follows:

**Qualitative Test of the Purity of the Phloroglucinol.**—Dissolve a small quantity of the phloroglucinol in a few drops of acetic anhydride, heat almost to boiling, and add a few drops of concentrated sulphuric acid. A violet colour indicates the presence of diresorcinol. A phloroglucinol which gives more than a faint coloration may be purified by the following method: Heat in a beaker about 300 c.c. of hydrochloric acid (sp. gr. 1.06) and 11 grm. of commercial phloroglucinol, added in small quantities at a time, stirring constantly until it has almost entirely dissolved. Some impurities may resist solution, but it is unnecessary to dissolve them. Pour the hot solution into a sufficient quantity of the same hydrochloric acid (cold) to make the volume 1.5 litres. Allow it to stand at least overnight—better several days—to allow the diresorcinol to crystallise out, and filter immediately before using. The solution may turn yellow, but this does not interfere with its usefulness. In using it, add the volume containing the required amount to the distillate.

**Procedure.**—Place a quantity of the material, chosen so that the weight of phloroglucide obtained shall not exceed 0.300 grm., in a flask, together with 100 c.c. of 12% hydrochloric acid (sp. gr. 1.06), and several pieces of recently heated pumice stone. Place the flask on a wire gauze, connect with a condenser, and heat, rather gently at first, and so regulate as to distil over 30 c.c. in about 10 minutes, the distillate passing through a small filter-paper. Replace the 30 c.c. driven over by a like quantity of the dilute acid, added by means of a separating funnel in such a manner as to wash down the particles adhering to the sides of the flask, and continue the process until the distillate amounts to 360 c.c. To the completed distillate gradually add a quantity of phloroglucinol (purified if necessary) dissolved in 12% hydrochloric acid, and thoroughly stir the resulting mixture.

The amount of phloroglucinol used should be about double that of the furfural expected. The solution first turns yellow, then green, and very soon an amorphous greenish precipitate appears, which grows rapidly darker, till it finally becomes almost black. Make the solution up to 400 c.c. with 12% hydrochloric acid, and allow it to stand overnight.

Filter the amorphous black precipitate into a weighed Gooch crucible through an asbestos felt, wash carefully with 150 c.c. of water in such a way that the water is not entirely removed from the crucible until the very last, then dry for 4 hours at the temperature of boiling water, cool and weigh in a weighing bottle, the increase in weight being reckoned as furfural phloroglucide. To calculate the furfuraldehyde, pentose, or pentosan from the phloroglucide use the following formulæ given by Kröber:

(a) For weight of phloroglucide "a" under 0.03 gram.

$$\text{Furfural} = (a + 0.0052) \times 0.5170.$$

$$\text{Pentoses} = (a + 0.0052) \times 1.0170.$$

$$\text{Pentosans} = (a + 0.0052) \times 0.8949.$$

In the above and also in the following formulæ the factor 0.0052 represents the weight of phloroglucide which remains dissolved in the 400 c.c. of acid solution.

(b) For weight of phloroglucide "a" over 0.300 gram.

$$\text{Furfural} = (a + 0.0052) \times 0.5180.$$

$$\text{Pentoses} = (a + 0.0052) \times 1.0026.$$

$$\text{Pentosans} = (a + 0.0052) \times 0.8824.$$

(c) For weight of phloroglucide "a" from 0.03 to 0.300 gram.

$$\text{Furfural} = (a + 0.0052) \times 0.5185.$$

$$\text{Pentoses} = (a + 0.0052) \times 1.0075.$$

$$\text{Pentosans} = (a + 0.0052) \times 0.8866.$$

Methylpentoses (such as rhamnose) are estimated volumetrically in the same way as pentoses, the conversion into methylfurfural taking place quantitatively on distillation of a methylpentose with hydrochloric acid in a current of steam.

In a mixture of pentoses and methylpentoses, the total sugar is determined by distillation with hydrochloric acid; in a second portion of the sample the methylpentoses are precipitated with alcohol and

saturated aqueous baryta at  $0^{\circ}$ , and the pentoses are estimated in the filtrate.

The method of estimating the furfural obtained in the acid distillation of pentoses and pentosans, which depends on its reducing action on Fehling's solution, was first proposed by Flohil (*Chem. Weekblad.*, 1910, 7, 1057) and subsequently modified by Eynon and Lane (*Analyst*, 1912, 37, 37). The procedure is as follows:

A portion of the acid distillate, obtained as in the phloroglucide method described above, containing not more than 0.05 grm. of furfural, is neutralised with sodium carbonate, mixed with 20 c.c. of Fehling's solution (Soxhlet's modification, see page 392), and the whole made up to 100 c.c. with water in a flask of about 200 c.c. capacity. The flask is fitted with a vertical reflux condenser and heated on an asbestos card over a flame of such size that the liquid begins to boil in 12 to 15 minutes, boiling being continued thereafter for exactly 35 minutes. The precipitated cuprous oxide is filtered off in a Soxhlet tube, washed with water and alcohol, dried at  $110^{\circ}$  to  $115^{\circ}$ , cooled and weighed; if desired, the oxide may be reduced to metallic copper and weighed as such, though this is not necessary. Since an appreciable amount of cuprous oxide is precipitated in blank determinations under the above conditions, and since the amount so precipitated increases with the amount of sodium chloride present, the weight of cuprous oxide or copper actually obtained must be diminished by an amount as found from the following table. In the acid distillation process described under the phloroglucide method, a distillate containing about 10% of hydrochloric acid is obtained, and the amount of sodium chloride formed on neutralising the portion of the acid distillate used may be calculated with sufficient accuracy.

Weight of NaCl, grm.	Cu <sub>2</sub> O, mg.	Cu, mg.
0.0	5.5	4.9
5.6	9.3	8.3
8.0	10.5	9.3
10.4	11.7	10.4

After making the correction by means of the above table, the amount of furfural is found by interpolation from the following table:

Weight of furfural, grm.	Weight of $\text{Cu}_2\text{O}$ (corrected), mg.	Weight of Cu (corrected), mg.	Ratio: $\frac{\text{Furfural}}{\text{Cu}}$
0.0100	30.0	26.6	0.376
0.0200	57.0	50.6	0.395
0.0300	81.0	71.9	0.417
0.0400	103.5	91.9	0.435
0.0500	125.0	111.0	0.450

The reducing powers of pure arabinose and xylose under the standard conditions of Brown, Morris and Millar (page 397) have been estimated by Daish (*J. Agric. Sci.*, 1914, 6, 225). The values obtained are given in the tables below, and refer to the anhydrous sugars.

## REDUCING POWER OF ARABINOSE

$$[\alpha]_D^{20} = 102.2^\circ \quad (c = 6.80)$$

Milligrams, arabinose	Grm. $\text{CuO}$	Calculated divisor	Divisor from curve
10	0.0270	2.700	2.669
20	0.0540	2.700	2.654
30	0.0804	2.680	2.640
40	0.1064	2.660	2.625
50	0.1320	2.640	2.610
60	0.1570	2.617	2.595
70	0.1820	2.600	2.581
80	0.2060	2.575	2.566
90	0.2300	2.556	2.551
100	0.2540	2.540	2.536
110	0.2780	2.527	2.521
120	0.3020	2.517	2.507
130	0.3248	2.490	2.492
140	0.3476	2.483	2.477
150	0.3700	2.467	2.461
160	0.3920	2.450	2.447
170	0.4140	2.435	2.432
180	0.4360	2.422	2.417
190	0.4570	2.405	2.403
200	0.4780	2.390	2.381

## REDUCING POWER OF XYLOSE

$$[\alpha]_D^{30} = 18.78^\circ (c = 5.07)$$

Milligrams, xylose	Grm. CuO	Calculated divisor	Divisor from curve
10	0.0280	2.800	2.656
20	0.0540	2.700	2.638
30	0.0798	2.660	2.620
40	0.1040	2.600	2.602
50	0.1300	2.600	2.581
60	0.1540	2.583	2.563
70	0.1790	2.557	2.545
80	0.2030	2.537	2.526
90	0.2260	2.511	2.508
100	0.2490	2.490	2.490
110	0.2720	2.473	2.471
120	0.2940	2.450	2.453
130	0.3160	2.431	2.433
140	0.3380	2.414	2.415
150	0.3600	2.400	2.397
160	0.3810	2.381	2.378
170	0.4020	2.365	2.360
180	0.4230	2.350	2.341
190	0.4440	2.337	2.322
200	0.4640	2.320	2.304

It will be seen that the reducing powers of xylose and arabinose are almost identical. For practical purposes, when working with the unknown pentoses in plant extracts, it is probable that no large error will be incurred by taking as the divisor the average value for arabinose and xylose corresponding with the weight of CuO dealt with. The reducing powers of arabinose and xylose differ, too, only slightly from that of dextrose; thus the divisors for these three sugars for 100 mg. of sugar are respectively 2.536, 2.490 and 2.538.

Pentoses are generally present in appreciable quantity in the solutions obtained by extracting foliage leaves with alcohol; when such solutions have been treated with basic lead acetate in the usual way, and the excess of lead has been removed by means of sodium carbonate, or other precipitant, these pentoses exercise a reducing action on Fehling's solution. When, therefore, it is necessary to make an analysis of such material, allowance must be made for these sugars, as indicated in the scheme given on page 501, before it is possible to calculate the proportion of other reducing sugar present, such as dextrose and lævulose (Davis and Daish, *J. Agric. Sci.*, 1914, 5, 465).

Kluyver (*Biochemische Suikerbepalingen*, 1914, page 181), considers that pentoses in the free state do not usually occur in plant extracts, but Davis and Sawyer (*J. Agric. Sci.*, 1914, 6, 406) have since given definite proof of their presence in extracts of certain leaves (turnip, mangold). Kluyver emphasises the fact that when other sugars, such as sucrose, lævulose, etc., are present, the values obtained by the ordinary Tollens-Kröber method of estimating pentoses by distillation with hydrochloric acid are high, owing to the formation of a furfural-like substance (probably hydroxymethylfurfuraldehyde) which yields an insoluble phloroglucide; the presence of pentoses might, therefore, be inferred in cases when these sugars were really absent, owing to the action of the hydrochloric acid used on sugars such as sucrose, dextrose and lævulose which were present in relative excess. Kluyver also suggests that the pentose estimation should only be carried out after fermenting away the other sugars. Davis and Sawyer, however, show (*loc. cit.*) that the error caused by the presence of these in estimating the pentoses in plant extracts is relatively small, and for most practical purposes can be neglected.

Cunningham and Dorée (*Biochem. J.*, 1914, 8, 438) have discussed at some length the formation of hydroxymethylfurfuraldehyde from various carbohydrates. They show that the condensation which produces furfural in the case of pentoses or pentosans takes place rapidly, and is almost completed before the hydroxymethyl-derivative begins to distil over. By using aniline acetate test paper it is possible to distinguish between the separation of the two aldehydes, and practically no error is made in pentosan estimations if this indicator is used. The hydroxymethylaldehyde is produced at a very slow rate. Its total amount is small, varying between 1 and 2% in the case of the hexoses, and those celluloses which contain little or no pentosan. The probable reason for this is the ready hydrolysis of the hydroxymethylfurfuraldehyde to formic and lævulic acids. This property explains the well-known observations of Fraps (*Amer. Chem. J.*, 1901, 25, 201), who found that when the distillates obtained from a number of natural products, food stuffs, etc., were distilled again, a considerable loss of "furfural" occurred. With pure furfuraldehyde this did not occur. He considered that the portion which disappeared could not be regarded as due to true pentosan, and in the absence of further

knowledge, designated it as the "furaloid" constituent. In all probability the "furaloid" is a hexose-yielding constituent which gives hydroxymethylfurfuraldehyde, and this, on a second distillation, is largely decomposed.

The formation of the hydroxymethylaldehyde, under the conditions given above, makes estimations of methylpentosans, by the method of Ellet and Tollens (*J. Landwirth*, 1905, 53, 13) of doubtful value.

**Estimation of Carbohydrates in Plant Extracts.**—The following scheme for the analysis of plant material such as foliage leaves, seeds, etc., has been suggested by Davis and Daish (*J. Agric. Sci.*, 1913, 5, 437).

The material, immediately after picking, is dropped into a large volume (2 litres) of boiling alcohol to which a little ammonia has been added (10 to 20 c.c. of ammonia, sp. gr. 0.880). This treatment destroys all the enzymes which are present and prevents change of the sugars during subsequent treatment.

The plant material is extracted in a large metal Soxhlet extractor for 18 hours. The extract is then evaporated *in vacuo* (60 to 20 mm.) to a small bulk and made up to a definite volume, e. g., 500 c.c. Of this, two portions of 20 c.c. each are evaporated to dryness and dried *in vacuo* for 18 hours at 100°. This gives the total dry matter in the extract. 440 c.c. are treated with the requisite volume of basic lead acetate solution, filtered under pressure on a Buchner funnel, the precipitate washed and the filtrate made up to a known volume (2 litres). This is called Solution A.<sup>1</sup>

300 c.c. of Solution A are freed from lead by means of solid sodium carbonate, any large excess being avoided, and made up to 500 c.c. This is called Solution B.

1. 25 c.c. of B are used for the *direct reduction* and *polarisation*;<sup>2</sup> the reduction is due to dextrose, lævulose, maltose, pentoses.

<sup>1</sup> It is often preferable when the solutions contain relatively small quantities of sugars to add sodium carbonate (or sodium sulphate) to remove the excess of lead before completing the volume to 2 litres. In such cases solution A, after filtering, is used directly for the sugar estimations.

<sup>2</sup> The polarisation of these dilute solutions is usually small, and it is therefore necessary to take the reading in a long tube (at least 200 mm. in length) with an instrument reading accurately to  $\frac{1}{100}^\circ$ , the temperature being maintained constant at 20° within  $\frac{1}{10}^\circ$ . It is an easy matter, using a Lowry thermo-regulator and circulating the water by means of a small pump, to keep the temperature constant to  $\frac{1}{100}^\circ$ , but differences of temperature less than  $\frac{1}{10}^\circ$  hardly make a perceptible difference in the readings with such dilute solutions as these.

If (as is usually the case) amides and amidic acids are present, which have an optical rotatory power, it is advisable to make the solution acid before measuring the rotatory power; for this purpose sulphur dioxide should be passed through the solution, as suggested by Pellet (see p. 380).

## 2. For Sucrose.—Invert 50 c.c. of *B*:

(a) By invertase. Make neutral to methyl orange by adding a few drops of concentrated sulphuric acid, add 1–2 c.c. of autolysed yeast and 2 or 3 drops of toluene, and leave 24 hours at 38° to 40°. After this period, add 5 to 10 c.c. alumina cream, filter and wash to 100 c.c. Take the reducing power of 50 c.c. (= 25 c.c. *B*) and polarise.

(b) By 10% citric acid. Make faintly acid to methyl orange by adding a few drops of concentrated sulphuric acid, and add a weighed quantity of citric acid crystals so as to have 10% of the crystalline acid ( $C_6H_8O_7 + H_2O$ ) present. Boil 10 minutes, cool, neutralise (to phenolphthalein) with sodium hydroxide, make up to 100 c.c., and determine the reducing power of 50 c.c. (= 25 c.c. *B*); polarise in faintly acid solution,

**Sucrose** is calculated from the increase of reducing power or change of rotation caused by inversion. The values obtained by the two methods (a) and (b) should agree closely.

3. For Maltose.—Another 300 c.c. of Solution *A* is freed from lead by means of hydrogen sulphide and filtered, the precipitated sulphide being washed until the total volume of filtrate and washings is about 450 c.c. Air is then drawn through this for about 1.5 hours to expel hydrogen sulphide, a very little ferric hydroxide is added to remove the last traces of the gas, and the solution is made up to 500 c.c. It is filtered and

50 c.c. fermented (a) with *S. marxianus*  
 50 c.c. fermented (b) with *S. anomalus*  
 50 c.c. fermented (c) with *S. exiguus*

and two lots *d* and *e* of 50 c.c. are fermented with bakers' yeast. It is generally necessary, in order to ensure good growth of the yeast, to reduce the acidity by adding 2 to 5 c.c. of N-sodium carbonate to the 50 c.c. to be fermented; 5 c.c. of sterilised yeast water are also added, the mixture is sterilised in the usual way and inoculated in the inoculating chamber with the pure culture of yeast. It is then stoppered with cotton wool, and the yeast allowed to incubate for 21 to 28 days at 25°.

After completion of the fermentation, 5 c.c. of alumina cream are added, the solution made up to 100 c.c. at 15° and filtered, and 50 c.c. are used for the reduction. The difference between the average reduction with *a*, *b* and *c* and the average of *d* and *e* gives the reduction due to *maltose*.

4. **Pentoses.**—These are approximately estimated in 50 c.c. of *A* by distilling with hydrochloric acid according to the Tollens-Kröber method (see p. 498).

5. When the reduction in (1) due to pentose and maltose has been allowed for, using the values for the reducing power of the pentoses given by Daish (see p. 501), the remaining reducing power is due to dextrose and lævulose; the actual proportions of these sugars, *in the absence of other substances possessing a rotatory effect on polarised light*,<sup>1</sup> can be calculated from the reducing power of the solution, combined with its corrected specific rotatory power, as suggested by Brown and Morris (*Trans. Chem. Soc.*, 1893, 63, 604).

A simple apparatus for evaporating plant and animal extracts *in vacuo*, such as is necessary in the above scheme of analysis, has been described by W. A. Davis (*J. Agric. Sci.*, 5, 434; *Chem. World*, 1914, 3, 239). By means of this apparatus all the difficulty encountered with such material, owing to the tendency to frothing which usually occurs, is completely overcome. Large volumes of liquid can be evaporated continuously and the distillate recovered, if necessary in fractions; the apparatus requires practically no watching after the distillation has once been started and can be left to itself while other work is proceeded with. It is only necessary from time to time to renew the liquid in the distilling flask *A*, by means of the dropping funnel *A'*.

The apparatus consists of an ordinary distilling flask with the side-tube bent up and passing into a wide piece of glass tube *B* which serves as a froth trap; this is connected by glass tubing with the condenser *D*, the lower end of which passes through a rubber stopper into the cylindrical dropping funnel *E*, which in turn is connected, as shown, below with the pump flask *G* and above with the large reservoir *P*, which serves to take up small variations of pressure and thus ensure a steady vacuum throughout the system. In this way regular boiling, without overheating or frothing, is secured.

The vacuum is maintained by means of an ordinary water injector pump, connected through a Hutchinson regulating valve *J* (*Chem-News*, 1912, 106, 99) with the bottle *H* and thence with *E* and *G*. a glass cock is interposed at *T*, whilst *S* is a screw-clamp which oper-

<sup>1</sup> Such substances are asparagine, glutamine, aspartic and glutamic acids, which occur widely in plant material and are not removed in the ordinary process of treatment with basic lead acetate, etc.; these substances are likely to cause error in the estimation of sugars in plant extracts and in materials such as beet-sugar molasses.

ates on the piece of rubber pressure-tubing connecting *G* and *H*. At *M* a manometer tube is inserted which shows the vacuum throughout the system. The Hutchinson valve takes up large variations in the vacuum due to changes of water pressure, so that by means of this, combined with the regulating reservoir *P*, changes in the vacuum are reduced to a minimum.

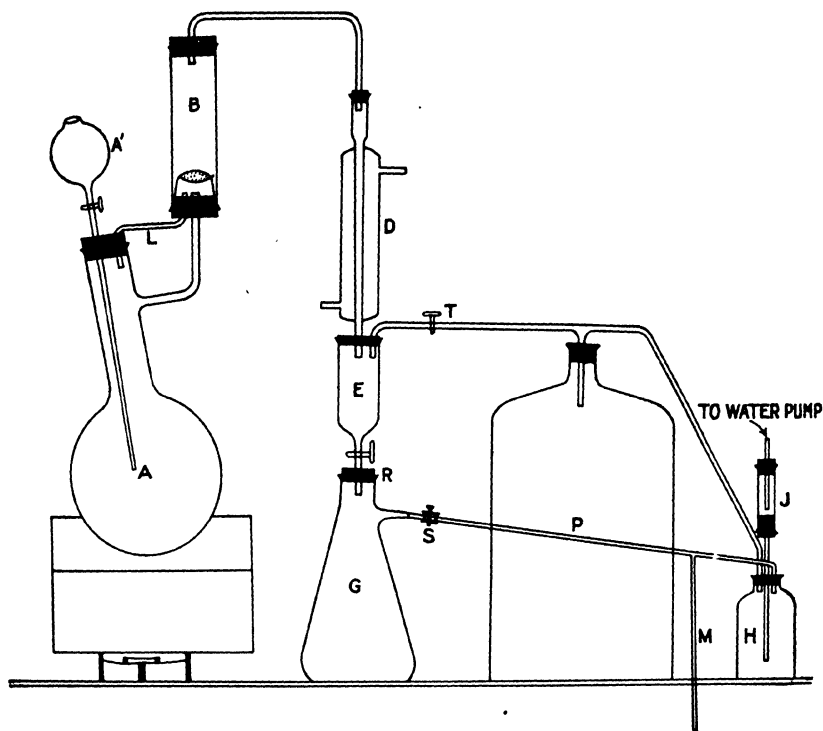


FIG. 65.

When the liquid in *A* first begins to boil there is often a great tendency to froth; should this occur, the froth rises into the trap *B*, breaks against a disc of copper-gauze, and the liquid is returned automatically to the flask through the piece of glass tube *L*.

The combination *E* and *G* allows of the distillate being removed from time to time; while the distillation is proceeding, the vacuum in *G* is maintained at the same degree as in the rest of the system so that by opening the glass tap of *E* the distillate runs down into *G*. When

*G* is full and it is required to empty it, the cock *E* is closed and the screw-clamp of *S* screwed down to the rubber pressure tube. This tube is then detached from the side tube of *G*, and the flask *G* removed from the rubber stopper *R*, emptied and replaced, without interfering with the vacuum throughout the rest of the system. After it has been replaced, *S* is opened, and in a very short time the vacuum is re-established in *G*, at the same degree as throughout the rest of the apparatus.

It is a simple matter, by introducing T-pieces, to run two or more of these distilling apparatus in conjunction with a single vacuum pump and a single regulating vessel *P*. All connections must, of course, be made with rubber stoppers or rubber pressure tubing.

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# STARCH AND ITS ISOMERIDES

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In the vegetable kingdom, and to a minor extent in the animal kingdom, there exist a number of carbohydrates having in common a composition represented by the empirical formula  $C_6H_{10}O_5$ , although their physical and chemical characters point in all cases to a multiple of this formula as the true representation of the magnitude of the molecule.

The carbohydrates of the starch group are non-volatile substances and, with perhaps one or two exceptions, amorphous substances. As a class they are insoluble in alcohol, though the greater number of them are dissolved by water, forming solutions which usually exert marked rotatory action on a ray of polarised light. They are neutral in reaction, and form but few definite compounds or metallic derivatives. They are very numerous, and apparently capable of isomeric modification. Owing to their physical characters and feebly marked chemical affinities, it is often difficult to obtain them in a state of purity.

None of the members of the group reduces Fehling's solution when boiled with it. By treatment with acids or by other means they undergo hydrolysis, the products formed include sugars which are able to reduce the cupric solution.

Many of the members of the group are of little practical interest, and their analytical reactions have been very incompletely studied. The following table serves to show the comparative characters of the more important members, cellulose, starch, and dextrin being described more fully in subsequent sections.

In general, starch and the other substances here considered are capable of exhibiting what are known as colloidal properties, one of the most marked of these being that such substances show, in solution, very little, if any, power of diffusion. The cause of this behaviour, which distinguishes substances in the colloidal, from those in the crystalloidal, state is the relatively great magnitude of the particles of the former distributed throughout the solvent. Some compounds are able in solution to show widely varying

“degrees of dispersion” and thus to form both colloidal and ordinary solutions. By means of the ultramicroscope, which, with the help of a bright beam of light passing through the liquid, shows up the particles existing in colloidal solutions as so many highly illuminated points in brisk motion, these particles may be measured and have been found to have dimensions lying between  $0.1\mu$  and  $1\mu$ , *i. e.*, between  $0.0001$  and  $0.000001$  mm.

Colloidal solutions are mostly far more unstable than true solutions, the dissolved substance readily separating either in flocks (coagulation) or as a stiff jelly (gelatinisation). According as the separated material (the so-called “gel”) is possessed or devoid of solubility in the solvent, this change is termed reversible or irreversible. Such precipitation, which often results in the simultaneous deposition of another colloid present in solution, is, on the other hand, sometimes prevented by the presence of another colloid. Many colloidal solutions, such as those of dextrans, starch, gums, pectinous matters, etc., are highly viscous.

With regard to their viscosity compared with that of the solvent or continuous phase, colloidal solutions divide themselves into two classes. Those of the one class, mainly colloidal metals and metallic sulphides, show viscosities but little greater than that of water, whereas those of the other, comprising principally the organic colloids, cause a very pronounced increase in viscosity, even if the percentage of dissolved matter is small. The former, owing to their similarity to a system composed of solid particles distributed throughout a liquid, are called suspensoids, whereas the latter resemble more closely systems composed of two liquid phases and are, hence, called emulsoids. This second class includes many important organic colloids, *e. g.*, starch, agar-agar, gums, cellulose, albumin, etc.

Various colloidal solutions or “sols”—as they are commonly called—with continuous phases other than water are known, chief among these being the sols of cellulose and of its nitrates, which are of great industrial importance as materials for producing artificial silk, etc. Solutions of cellulose in ammoniacal cupric oxide solutions form typical emulsoid sols of very high viscosity, whilst the solutions in 50% zinc chloride solution exhibit even higher viscosities.

For further information concerning colloidal solutions reference may be made to any of the text books dealing with this subject, *e. g.*,

Name	Empirical formula	Chief sources and modes of formation	Specific rotatory power	Solubility in water	Products obtained by boiling with dilute acid	Reaction with iodine solution	Other characters
Cellulose	$C_6H_{10}O_5$	Cotton-wool; filter-paper; linen-rags; woody fibre.	.....	Not soluble.	Not changed.	No change; blue in presence of zinc chloride.	Soluble in Schweitzer's reagent, forming levorotatory solution. With strong sulphuric acid, followed by dilution, gives dextrose, etc.
Starch	$C_6H_{10}O_5$	Amylaceous seeds, roots, etc.	$[\alpha]_D = +200^\circ$	Insoluble, cold; gelatinised and dissolved on boiling.	Maltose and dextrin; ultimately dextrose.	Violet-blue.	White powder of characteristic appearance under microscope. Insoluble in Schweitzer's solution. Precipitated by tannin and ammoniacal lead acetate.
Glycogen	$C_6H_{10}O_5$	Liver of man and herbivorous animals; yeast.	$[\alpha]_D = +107^\circ$	Slowly soluble; solution opalescent, cleared by acetic acid.	Dextrose.	Wine-red.	White amorphous substance, readily soluble in alkaline liquids.
Inulin	$C_6H_{10}O_5$	Elecampane; dahlia; dandelion; chicory; Jerusalem artichokes.	Levorotatory.	Cold, slightly soluble; hot, readily soluble.	Lævulose.	No change.	White, hygroscopic powder, or sphaerocrystals. Insoluble in absolute, sparingly in dilute, alcohol. Reduces silver nitrate solution.
Dextrin	$C_6H_{10}O_5$	Action of acids or diastase on starch.	$[\alpha]_D = +200^\circ$	Readily soluble.	Dextrose.	Erythrodextrin, reddish-brown; achroo-dextrin, colourless.	White, very deliquescent. Apparently two varieties, differing in their reaction with iodine. Insoluble in alcohol.
$\alpha$ -Amylan	$C_6H_{10}O_5$	Barley, etc.	$[\alpha]_D = -21.6^\circ$	Cold, nearly insoluble; hot, gelatinises and dissolves sparingly.	Dextrose.	No change.	Amorphous, white substance.
$\beta$ -Amylan	$C_6H_{10}O_5$	Wheat, rye, etc	$[\alpha]_D = -129.7^\circ$	Soluble in cold water, forming very viscous solution.	A dextroglucose.	No change.	Amorphous, white substance. Solution exhibits mutarotation.
Gums	.....	.....	Lævorotatory.	Soluble in, or swollen by, cold water.	Hexoses and pentoses.	No change.	Amorphous. Solutions highly colloidal. Insoluble in alcohol. Yields mucic acid on treatment with nitric acid.

Hatschek's "*An Introduction to the Physics and Chemistry of Colloids*" (London, 1922), W. W. Taylor's "*The Chemistry of Colloids*" (London, 1916), and E. F. Burton's "*The Physical Properties of Colloidal Solutions*" (London, 1916). The applications of colloids to technical chemistry are dealt with in "*Die Bedeutung der Kolloide für die Technik*" (Dresden, 1909) by K. Arndt, in "*Colloid Chemistry and some of its Technical Aspects*" by J. Alexander (*J. Soc. Chem. Ind.*, 1909, 28, 280), in four "*Reports on Colloid Chemistry and its Industrial Applications*" (British Association for the Advancement of Science, 1918; H. M. Stationery Office, 1919, 1920 and 1922), and in "*Colloidal Chemistry and Brewing*" by E. Hatschek (*J. Fed. Inst. Brewing*, 1912, 18, 494).

## STARCH

Starch is one of the characteristic products of the vegetable kingdom and is formed in almost all organs of plants. It is a white, glistening, tasteless powder, fixed in the air and not volatile or crystallisable. It is very hygroscopic and contains as much as 20% or more of moisture<sup>1</sup> when air-dried and still about 10% when dried in a vacuum.

Starch is not dissolved without change by any known solvent and is quite unacted on by alcohol, ether or cold water. When heated with water to a temperature which varies slightly according to the origin of the starch,<sup>2</sup> the granules swell up and form a paste, or, in presence of much water, a colloidal solution. It is more than probable that starch is not a single substance of very high molecular complexity, as is generally supposed, but a mixture of closely related isomerides of comparatively simple structure.

According to Karrer and Nägeli (*Helv. Chim. Acta*, 1921, 4, 263) starch represents a polymerised maltose anhydride (diamylose), no polysaccharide higher than maltose being obtained by its hydrolysis; the degree of polymerisation of starch is probably not very high, the high value formerly attributed to its molecular weight being presumed erroneously from its insolubility. Glycogen also

<sup>1</sup> In our experience several of the most used varieties of starch in the U. S., such as wheat, corn [maize], tapioca, rice and potato starch, contain about 6 to 10% of moisture in an air-dry condition. Am. Eds.

<sup>2</sup> The gelatinisation temperatures are as follows:

Green malt and oat starch, .....	85°
Barley, kilned malt, rye, wheat and rice starch, .....	80°
Maize starch, .....	75°
Potato starch, .....	65°

is to be regarded as diamylose polymerised to a degree different from that of starch.

On hydrolysis with dilute acids, starch is converted into a mixture of dextrins and maltose, prolonged treatment resulting in further hydrolysis and ultimately in complete conversion into dextrose. Starch solution or paste is hydrolysed by malt extract (*diastase*) to dextrin and maltose even in the cold, but solid starch is not attacked by malt extract unless a liquefying enzyme is also present. The saliva enzyme (*ptyalin*) also hydrolyses starch.

**Soluble starch** may be produced by boiling starch with water, the solution obtained being rendered quite clear by the addition of a little alkali hydroxide. It is the first product of the action of dilute acids or amylolytic enzymes on starch. The method recommended for its preparation by the Malt Analysis Committee (see under Malt) consists in digesting purified potato starch with dilute hydrochloric acid (sp. gr. 1.037) at 15.5° to 18° (60° to 65° F.) for 7 days and washing the product free from acid by repeated treatment with water.

Soluble starch is a very perfect colloid and has a high viscosity. It is strongly dextrorotatory.

Parow, Ellrodt, and Neumann (*Zeitsch. Spiritusind.*, 1907, 30, 432) give the following mean results for the sp. gr. of various starches.

Starch from:	Sp. gr. of anhydrous starch in:		Sp. gr. of hydrated starch in:			
			Water		Toluene	
	Water	Toluene	% of water in the starch	Sp. gr.	% of water in the starch	Sp. gr.
Potatoes.....	1.648	1.513	18.72 19.35 20.14	1.463 1.436 1.453	15.03	1.361
Wheat.....	1.629	1.502	13.38 13.80 14.00	1.515 1.496 1.492	13.90	1.365
Maize.....	1.623	1.499	11.06 12.88 14.36	1.522 1.504 1.490	12.60	1.378
Rice.....	1.620	1.504	11.92 13.10 14.14	1.514 1.500 1.501	14.03	1.360

**Structure of Starch Granules.**—Starch occurs in plants in the form of minute granules, which generally possess a concentrically stratified structure similar to that of the onion. Starch granules are wholly unacted on by cold water, but when the outer layer of the granule is ruptured, as by grinding the starch with sand, water acts readily giving a liquid which yields an intense blue colour with iodine. This and other analogous phenomena gave rise to the view formerly held, namely, that the starch granule consists of an outer layer known as starch-cellulose and an inner material termed granulose. Mayer, however, considered the granule to be homogeneous, and stated that the thin films remaining after gelatinised starch has been treated with various reagents are formed by the action of such reagents and are identical with amyloextrin. Maquenne and Roux regard amylocellulose (or amylose) in various states of condensation as constituting the bulk (80–85%) of the granules, the rest being amylopectin, to which the viscosity of starch paste is due. Amylopectin is mucilaginous and gives no colour with iodine.

Amylose is gradually deposited from starch paste or solution, this reversion, giving what is known as artificial starch, being accelerated by an enzyme, amylocoagulase, contained in cereals. The reversion product appears to be wholly saccharifiable (amylose), whereas the original starch contains a more refractory substance (amylopectin) attacked comparatively slowly by diastase. It seems possible that amylopectin differs from amylose only in degree of condensation or polymerisation.

Young, small corpuscles of starch appear invariably to be spherical, but as they grow older they may become lenticular, ovoid, or polygonal. The shape and size of the starch granules are often highly characteristic of the plant by which they were produced, and this fact is frequently taken advantage of for determining the sources of particular starches.

According to Fernbach (*Compt. rend.*, 1904, 138, 428), small granules of starch (potato) consist of a nucleus relatively rich in phosphorus, layers of starch free from phosphorus being gradually superposed on this nucleus to form the larger granules.

**Microscopic Identification of Starches.**—When a sample is to be examined under the microscope for the identification of its starch, a minute quantity should be placed on a glass slide. With material either powdered or readily reducible to powder, a preferable plan

is to stir the sample with a dry glass rod, and tap the rod on the glass slide. A drop of distilled water or diluted glycerol (1 of glycerol to 2 of water) should then be added, and if the unpowdered substance be employed it should be broken up by careful crushing with the point of a knife. A cover-glass is then put on, and any superfluous moisture removed by blotting-paper. The specimen is now ready for observation. Somewhat oblique light should always be employed, and the magnification should be about 200 diameters.

The points to be observed in the microscopic observation of starches are: (a) The shape and size of the granules. (b) The position and character of the *hilum*,<sup>1</sup> round which are disposed (c) The concentric markings. (d) The appearance under polarised light. The first two observations are tolerably simple, but the examination for rings requires care, the markings being rarely visible without very cautious manipulation of the illumination and movement of the fine adjustment, and then in only a few granules at the same time. Natal arrowroot and turmeric starches show well-developed rings on nearly every granule. Wheat, on the other hand, shows few rings, even in the best light. When the hilum is situated near the centre of the granule, the rings are usually completely visible, but when the hilum is near one end of the granule only a segment of each ring is visible.

Although the size of starch granules is a highly useful character, it must be remembered that great differences are often noted between individual granules, and that it is only the general or average size which is usually recorded. With potato starch, these differences are very marked, the granules ranging from 0.0025 in. in length down to less than 0.0002 in. (0.063 mm. to less than 0.005 mm.).

Examination with polarised light, either with or without the help of a selenite plate, is a valuable auxiliary means of identifying starches, but many of the statements made in books, for instance, that the black cross is observable in the case of certain starches only, must be considered as applicable merely to the precise conditions under which the observations referred to were made. With proper manipulation, all starches appear to show the black cross, and ignorance of this fact has led many into error. Some starches

<sup>1</sup> According to Reychler, two hila exist, one on each side of the starch granule.

show much more colour than others when examined under the polarising microscope. For observation of starches by polarised light it is often desirable to employ a highly refracting mounting medium, and for such purposes water may be advantageously replaced by diluted glycerol, glycerol jelly, Canada balsam, oil of anise, carbon disulphide, etc.

Much has been written on the microscopic appearance of starches, and some observers profess to be able to distinguish starches of almost every origin. To the observer who has not made a special study of the morphology of starches, the distinctions are in many cases wholly unrecognisable, and as the minute differences are almost incapable of either description or delineation, the only safe method of discriminating starches is by a careful comparison of the sample with specimens of known origin and purity, the observations being made under exactly similar conditions as to illumination, magnifying power and mounting medium. These standard specimens should be not permanently mounted, but kept in an air-dry state, and a minute quantity mixed with water or other medium when required for use. As a rule, it is quite unnecessary to prepare the pure starches for comparison, direct employment of the air-dried tissue answering every purpose.

Very complete tabular schemes for the recognition of starches by the microscope have been devised by Muter (*Organic Materia Medica*), and this subject is treated also by Greenish in his *Microscopical Examination of Foods and Drugs*, by Galt in *Microscopy of the Starches* (London, 1900) and by other authors. Descriptions and diagrams do not enable the observer to dispense with the requisite experience, but they facilitate the recognition by drawing attention to the more characteristic features of the starches. The figures of starch granules on pages 520 to 522 are derived from Villavecchia's *Applied Analytical Chemistry*, Vol. II (London, 1918), the magnification being 200 in each case.

The following arrangement of starches, according to their microscopic appearance, is based on that of Muter. The starches are arranged in 5 classes.<sup>1</sup>

<sup>1</sup> In order that mistakes may not be made in differentiating starches by the scheme, it is important to bear in mind that the appearances described apply to the following conditions of examination, namely, observation with oblique light, use of water as a medium, and, when polarised light is used, the use of a red-green selenite plate with diluted glycerin as a mounting medium.

I. **The potato group** includes such oval or ovate starches as give a play of colours when examined by polarised light and a selenite plate, and have the hilum and concentric rings clearly visible.

II. **The leguminous starches** comprise such round or oval starches as give little or no colour with polarised light, have concentric rings all but invisible, though becoming apparent, in many cases, when the starch is treated with chromic acid, while the hilum is well marked, and cracked or stellate.

III. **The wheat group** comprises round or oval starches having both hilum and concentric rings invisible in the majority of the granules. It includes the starches from wheat and some other cereals, and starches from a variety of medicinal plants, such as jalap, rhubarb, senega, etc.

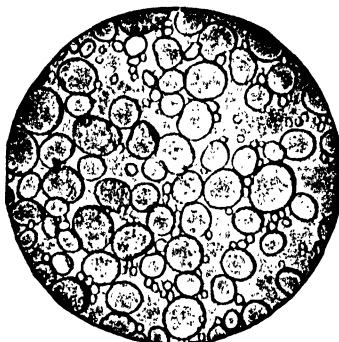


FIG. 61.—Wheat starch.

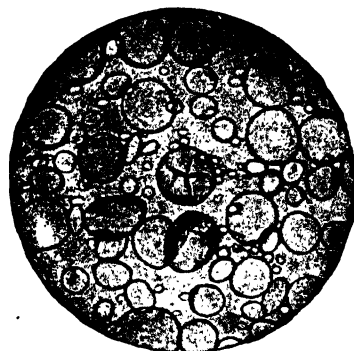


FIG. 62.—Rye starch.

IV. **The sago group** comprises starches of which all the granules are truncated at one end. It includes some starches used for food, together with those from belladonna, colchicum, scammony, podophyllum, canella, aconite, cassia, and cinnamon.

V. **The rice group** contains starches all the granules of which are polygonal in form. It includes those from oats, maize, buckwheat, rice, pepper, and ipecacuanha.

The following table gives further particulars respecting the microscopic appearance of the more important starches. The figures expressing the sizes are micromillimetres ( $1/1000$  mm.), and may be converted into ten-thousandths of an inch by multiplying them by the factor 0.3937.

In the case of elongated starches, the figures expressing the size have reference to the mean of the longer and shorter diameters.

Origin of starch	Diameter in micro-millimetres	Characteristic shape of granules	Other characters
<b>CLASS I</b> Canna, or <i>tous-les-mois</i> .	47-132	Irregular oval, or oyster-shaped.	Hilum annular and eccentric. Rings incomplete, very fine, narrow, and regular. Alkali develops lines and hilum. Well-marked and regular cross with polarised light.
Potato.	Very variable; usually between 60 and 100	Small granules, circular; the larger ovate, or oyster-shaped. (Fig. 68.)	Hilum, a spot, generally near smaller end. Rings in larger granules numerous and complete. Very distinct cross towards smaller end, and brilliant colours, with polarised light.
Maranta arrowroot.	10 to 70, average 36	Somewhat ovoid or mussel-shaped, tending to triangular in larger granules. Sometimes irregular, with a nipple-like projection at same end as hilum. (Fig. 70.)	Hilum, near one end, either circular or linear, and often cracked. Rings numerous and always visible, but not strongly marked. Well-defined cross towards larger end with polarised light, and brilliant colours.
Natal arrowroot.	33 to 38	Broadly ovate, or occasionally circular, with irregular projections.	Hilum, a crack, eccentric. Rings very distinct under water.
Curcuma or East Indian arrowroot.	30 to 61	Resembles maranta. Elongated, or oval with irregular projection. (Fig. 73.)	Hilum, an eccentric dot or circle. Indistinct segments of rings. Heat or alkali deforms granules very irregularly.
<b>CLASS II</b> Bean.	Nearly uniform 30 to 35	Reniform or oval. (Figs. 65 and 86.)	Hilum, stellate, often becoming a longitudinal furrow. Smaller granules predominate.
Pea.	Very variable 20 to 40	Reniform or oval. (Fig. 66.)	Hilum elongated. Not distinguishable from bean in mixtures.
Lentil.	20 to 40	Reniform or oval. (Fig. 67.)	Hilum elongated and very clearly defined. Rings moderately distinct.
<b>CLASS III</b> Wheat.	Very variable 2 to 52	Circular or nearly so, and flattened. (Fig. 61.)	Chiefly of two sizes, large and very small. Shows a cross in glycerol with polarised light, but very slightly in water. Faint rings and colours are visible on most of the elliptical granules.
Barley.	Fairly uniform 13 to 39	Closely resembles wheat; some granules slightly angular, or elliptical. (Fig. 63.)	Not distinguishable with certainty from wheat in mixtures of the two.

Origin of starch	Diameter in micro-millimetres	Characteristic shape of granules	Other characters
Rye.	2 to 38	Closely resembles wheat. (Fig. 62.)	A few granules show a three- or four-armed fissure extending nearly to the circumference.
Oat.	.....	Large oval granules showing polygonal divisions. (Fig. 85.)	The compound granules break up by attrition into polygonal granules (see Class V).
Acorn.	19	Circular or slightly oval.	Eccentric hilum developed by chromic acid.
<b>CLASS IV</b>			
Arum.	14	Truncated with two facets.	Hilum eccentric.
Tacca arrow-root.	9 to 19	Resembles tapioca.	Distinct hilum, linear and often starred. Very varied shape, often resembling maize, but with sharp angles.
Sago.	25 to 66	Ovate, or truncated oval. (Fig. 71.)	Hilum, a circular spot or crack at convex end; faint rings. Well-defined cross and often colours with polarised light. <i>Prepared sago</i> shows large oval depression; with polarised light characters less definite than with raw sago.
Tapioca.	8 to 22	Kettle-drum, or circular.	Hilum, a dot or short slit, nearly central. Well-defined cross and colours with polarised light. Characters of <i>prepared tapioca</i> are less definite.
<b>CLASS V</b>			
Rice.	5 to 8	Pentagonal or hexagonal, occasionally triangular. (Fig. 84.)	Angles sharply defined. Distinct hilum under a very high power, and cross visible in larger granules with polarised light.
Buckwheat.	5 to 20 depending on variety	Resembles oat and rice, but angles more rounded. (Fig. 82.)	No rings, but distinct central hilum, as spot or star. Well-defined cross, with polarised light. Granules often compound.
Oat.	4 to 30	Triangular to hexagonal, a few small and round or apple-pip-shaped. (Fig. 85.)	Rings and hilum invisible except under very high powers. Faint cross with polarised light.
Maize.	7 to 20	Circular to polyhedral, usually with rounded angles. (Fig. 64.)	Hilum central, as a well-defined star or crack. Rings nearly invisible. Distinct cross and faint colours with polarised light.
Dari, or Dhurra	19	Small elongated hexagons. (Fig. 83.)	
Pepper.	½ to 5	Resembles rice, but majority decidedly smaller.	Shows hilum with very high power. Granules often in motion. Forms large compound granules of very irregular form.

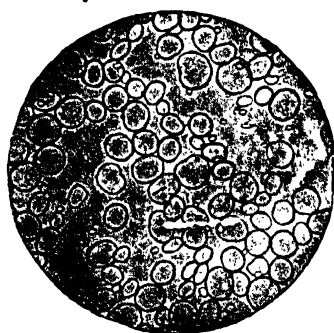


FIG. 63.—Barley starch.

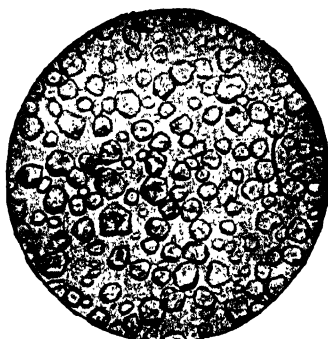


FIG. 64.—Maize starch.

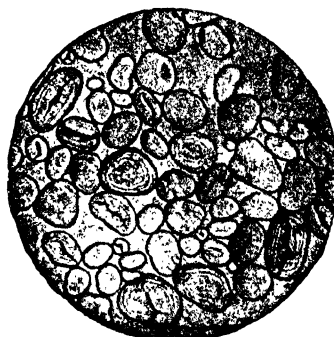


FIG. 65.—Broad bean starch.

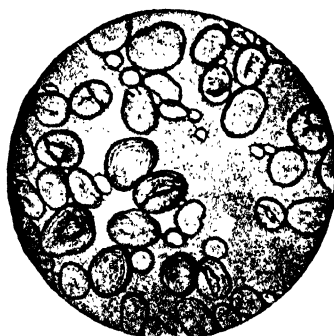


FIG. 66.—Pea starch.

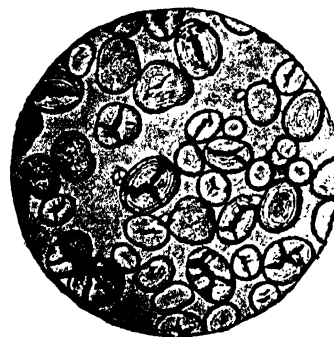


FIG. 67.—Lentil starch.

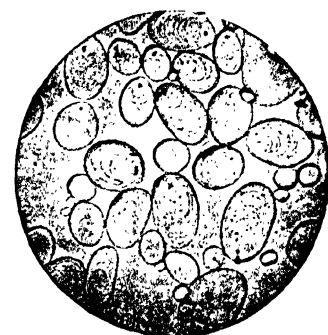


FIG. 68.—Potato starch.



FIG. 69.—Sweet potato starch.

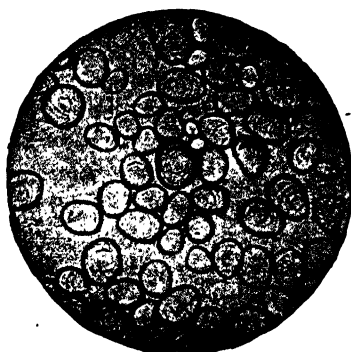


FIG. 70.—Maranta (arrowroot) starch.



FIG. 71.—Sago starch.

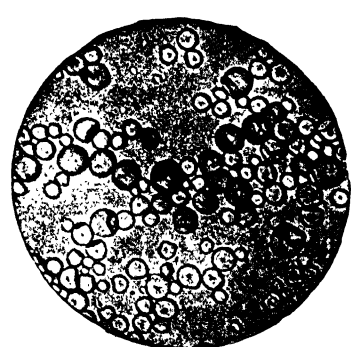


FIG. 72.—Manioc starch.



FIG. 73.—E. Indian arrowroot starch.

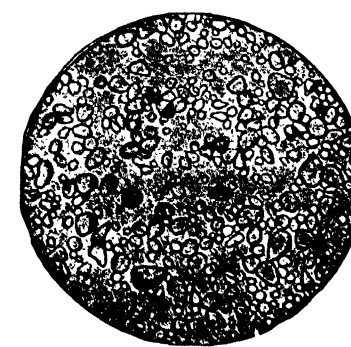


FIG. 74.—Chestnut starch.

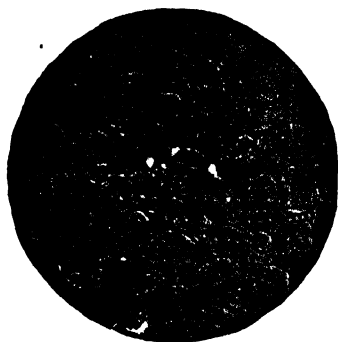


FIG. 75.—Starch from loaf made with an 80% flour.



FIG. 76.—Starch from a small fancy loaf.

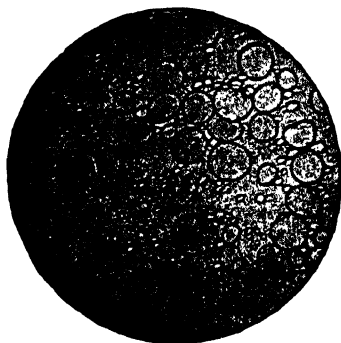


FIG. 77.—Starch from wheaten macaroni.

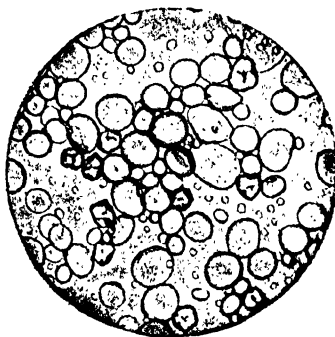


FIG. 78.—Starch from mixed wheat and maize macaroni.

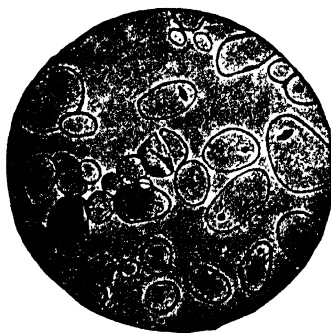


FIG. 79.—Dextrin from potato starch.



FIG. 80.—Ivory-nut meal.

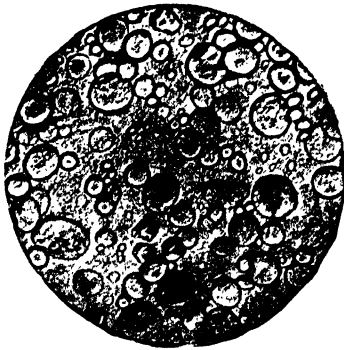


FIG. 81.—Mixture of wheat and maize starches.

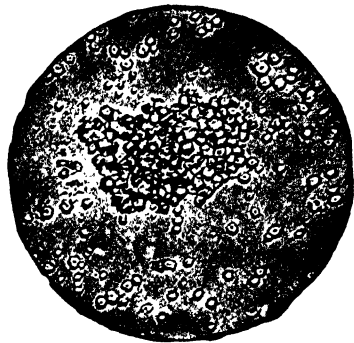


FIG. 82.—Buckwheat starch.

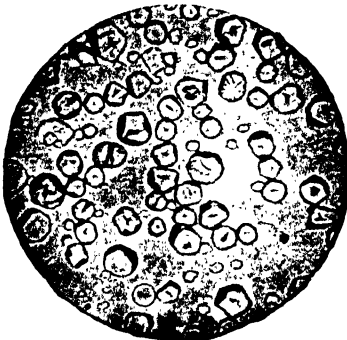


FIG. 83.—Dhurra starch.

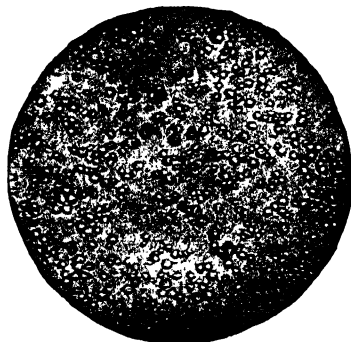


FIG. 84.—Rice starch.

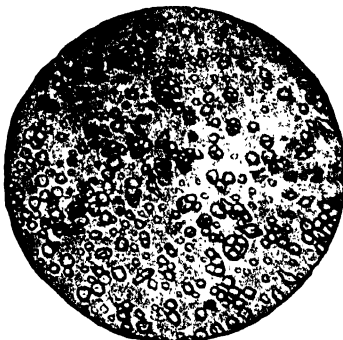


FIG. 85.—Oat starch.

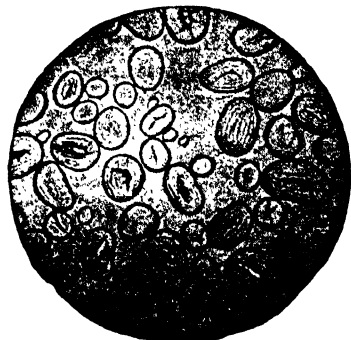


FIG. 86.—French bean starch.

**The arrowroot** of commerce is the starch derived from plants of the genus *Maranta*, belonging to the order *Marantaceæ*. For trade purposes arrowroot is distinguished by the name of the island or country producing it.

The starch granules of the different species and varieties of *Maranta* differ considerably in their microscopic appearance, while certain varieties are closely simulated by the starches from quite different plants. This is the case, for instance, with the starch of *Curcuma angustifolia*, sometimes called East Indian arrowroot.

Arrowroot is liable to adulteration with a variety of cheaper starches, though the practice is far less common now than formerly. The principal starches which have been employed, either as substitutes for arrowroot or for mixing therewith, are those of potato, sago, tapioca, curcuma, and tous-les-mois. Tacca and arum starches are also stated to have been employed, but are not known at present in British markets.

The microscope affords the only satisfactory means of distinguishing maranta starch from the starches above mentioned, and even then the detection of certain admixtures is a matter of considerable difficulty. *Potato*<sup>1</sup> and *tous-les-mois* starches are distinguished by their large size and regular and well-developed concentric rings, and potato, in addition, by the hilum being situated near the smaller end of the granule. *Sago*, *tacca*, *arum* and *tapioca* are distinguished by the truncation of the granules. *Curcuma* starch forms irregular oblong granules rounded at one extremity but often tapering rather abruptly at the other. The grains are so flat that when viewed on their edges they appear rod-shaped.

**The cereal starches** may be divided into two well-defined groups, wheat, barley and rye starches being circular or nearly so, whilst the starches of rice, maize, buckwheat and oats are polygonal.

**The leguminous starches** present very close resemblances and are generally indistinguishable from each other when in admixture.

<sup>1</sup> Besides in its microscopical appearance, potato starch is said to be distinguished from maranta starch in the following respects: 1. When mixed with twice its weight of strong hydrochloric acid, maranta starch produces an opaque white paste, while the paste produced by potato starch is transparent and jelly-like. 2. Potato starch emits a peculiar odour when boiled with dilute sulphuric acid. 3. An acrid oil may be extracted from potato starch, but not from that of maranta.

**Proportion of Different Starches in Admixture.**—The following method<sup>1</sup> is the most suitable for ascertaining the extent to which oatmeal is mixed with barley or wheaten flour, and is a type of the process to be employed in other cases. Genuine pearl-barley is ground finely in a mortar, and a series of standards made by mixing the flour with definite proportions of genuine oatmeal. Mixtures containing 5, 10, 15, 20, 30 and 40% of barley, respectively will be found convenient in practice. The sample of oatmeal to be examined is thoroughly mixed, and 0.1 grm. weighed out and ground in an agate mortar with a little water. When the mixture is perfectly smooth it is rinsed into a small conical glass, and diluted with water to 10 c.c. Two of the standard mixtures (say the 10 and 20% mixtures) are then treated in a precisely similar manner. A drop of the sample and one of each of the standards are then placed on glass slides and covered with thin cover-glasses. Care must be taken that the starches and water are thoroughly agitated, so that the drops taken shall be representative, and it is important that the drops themselves shall be of exactly the same size. These conditions are best ensured by immersing in the liquid the lower portion of a piece of glass tubing drawn out to a fine point, blowing down it so as to mix the sample thoroughly by means of the air-bubbles expelled, and then allowing a drop of the liquid to fall from the orifice of the tube on to the glass slide. The same tube is then employed to take drops of the standard mixtures. The cover-glasses must be of equal size and sufficiently large to take up the whole of the drop, as none of the liquid must be removed. The slides being prepared, the number of barley granules visible in twelve successive fields is noted. The standards are then similarly observed, the operation being repeated until a standard mixture is found, the barley granules in twelve fields of which are equal or nearly equal in number to those counted in the sample. The proportion of wheat or barley in the sample will then be approximately the same as that in the standard with which it agrees.

<sup>1</sup> Dr. James Bell gives the following method for estimating starches in admixture: "The sample is first rubbed in a mortar and passed several times through a sieve. A small quantity, say 0.003 grm., is then weighed out and placed on a glass slide, where it is worked into a thin paste with about 2 drops of water. A thin cover glass, measuring about 1.5 in. by 1 in., is then placed over the paste, and moved about the slide until the paste is equally distributed and all under the cover-glass. The number of granules is counted in nine fields, representing as fairly as possible the entire slide. The process is repeated till a correct idea of the composition of the sample is obtained. Standard mixtures approximately representing the sample are made up and treated in exactly the same way, and from a comparison of the results the percentage of foreign starch is computed."

Winter Blyth (*Society of Public Analysts*, June, 1918) gives a method for identifying starches by taking careful measurements of the individual granules and from the results of these measurements compiling graphs, each starch having its own typical graph. The varying behaviour of different starches towards dyestuffs is used by Unna (*Zeitsch. Unters. Nahr.-Genussm.*, 1918, 36, 49) as the basis of a micro-colourimetric method for the identification of wheat, rye and potato starches in admixture.

Blunck (*Zeitsch. Unters. Nahr.-Genussm.*, 1915, 29, 246) finds that the dye known as Metachrome-Red G Agfa colours potato starch and cell tissues bright golden-yellow, but does not colour cereal starches. In applying this test to bread, the acidity of the latter must be first removed by treatment with dilute alkali solution and subsequent washing.

**Detection and Estimation of Starch.**—For the detection of starch existing in the *solid* state, no other method is so good as the microscopic recognition of the granules, the origin of which may usually be ascertained in the manner already described. The microscopic examination may be advantageously supplemented by adding a drop of iodine solution to the slide, when each of the true starch granules will assume a blue colour, which renders their recognition easy. In some cases, as when roasted coffee is mixed with beans or acorns, the microscopic detection of the starch becomes difficult, but may still be effected in the following manner: the coffee is boiled with water for a few minutes, and the solution is decanted or filtered from the insoluble matter. The liquid is next thoroughly cooled and cold dilute sulphuric acid is added. A solution of potassium permanganate is then gradually added till the brown colour is nearly or entirely destroyed, when the decolourised liquid is tested with iodine. A blue colour is obtainable in this way with coffee containing only 1% of roasted acorns.

Sometimes it is desirable to remove the colouring matter from the solid substance before examining it for starch. If cold water fail to effect this, alcohol should be tried, and subsequently other solvents. The cases are rare, however, in which the starch cannot be observed microscopically after successive treatments of the substance with cold water and alcohol.

In *aqueous solution*, starch yields with ammoniacal lead acetate a precipitate having a composition represented approximately by the

formula  $C_{12}H_{18}Pb_2O_{11}$ . Tannin gives with starch solution a white precipitate disappearing on warming and reappearing as the liquid cools. Soluble starch is completely precipitated by adding alcohol to its aqueous solution.

The most characteristic reaction of starch solution is the violet or indigo-blue colouration which it gives with iodine; the coloured substance formed appears to be not a definite compound of starch with iodine, or a solid solution of iodine in starch, but an adsorption compound in which widely varying proportions of starch and iodine may occur. The best form in which to employ the reagent is as a very dilute solution of iodine in potassium iodide, the starch solution being cold. When heated, the liquid is decolourised, but on cooling the blue is restored, though not with the original intensity. In employing the reaction as a test for starch it is necessary to remember that it is produced only by *free* iodine. Hence any free alkali should be neutralised by cautious addition of cold dilute acid, and any reducing or oxidising agent eliminated if possible. The best way of testing for starch is to add the iodine solution gradually to the slightly acid liquid until either a blue appears or the liquid is turned permanently yellow by the free iodine. If the latter effect is produced no starch can be present.

The only organic compound liable to interfere when the test is performed in the foregoing manner is erythrodextrin, this producing with iodine an intense reddish-brown colouration which is apt to mask a feeble starch reaction. The affinity of iodine for starch is, however, greater than its affinity for erythrodextrin, and hence if a very little iodine solution be employed the blue due to starch will alone be developed, the brown becoming apparent on further addition of the reagent. By cautious addition of very dilute ammonia or by gradually heating the liquid, the brown colour may be destroyed whilst the blue remains.<sup>1</sup>

## ESTIMATION OF STARCH

The accurate estimation of the starch in a natural product is by no means an easy matter, owing to the varied nature of the substances occurring with the starch and the difficulties involved in effecting the necessary separation. No method for estimating starch has, indeed, received general acceptance, and possibly no

<sup>1</sup> Neither the brown colour of a solution of iodised erythrodextrin nor the blue of iodised starch shows absorption bands when examined by the spectroscope.

method is capable of use with all starch-containing materials. Naturally most attention has been directed to the estimation in the case of cereal grains, potatoes, etc., the dry matter of which consists very largely of starch, although methods have also been devised for use with vegetable products containing comparatively low percentages of starch. The various methods given below represent those most commonly employed.

**Baumert and Bode's Method.**—This method is based on the fact that starch is readily soluble in aqueous sodium hydroxide but is insoluble in, and may be precipitated quantitatively from, alcoholic sodium hydroxide. Baumert (*Zeitsch. Unters. Nahr.-Genussm.*, 1909, 18, 167) gives the following modification of this method, applicable to all starches (wheat, rye, barley, oats, rice, maize): 3 gm. of the finest possible powder is evenly macerated in a beaker with 2 to 5 c.c. of water and mixed with 10 c.c. of hydrochloric acid (1.19) with continual stirring and cooling (by placing in cold water). To the gelatinous mass, which will have become fluid in 10 minutes at most, excess of sodium hydroxide (20%) is added, the mixture being well stirred and cooled. The contents of the beaker are then washed with water into a 250 c.c. flask, made up to the mark, well shaken, and after allowing to settle, filtered through a pleated filter. 25 c.c. of the filtrate, after addition of about 1 gm. of finely flocculated asbestos, is precipitated with 50 to 60 c.c. of alcohol (94 to 96%) whilst being vigorously stirred. As soon as the precipitate has quite settled, it is collected by the aid of suction on a previously ignited asbestos filter-tube, washed with alcohol containing 3 to 5 c.c. of dilute hydrochloric acid (to decompose the sodium-starch compound), then with 80% alcohol, again with absolute alcohol and finally with ether. After the tube has been dried and weighed, the contents are heated in a current of oxygen and the tube reweighed after cooling. The loss in weight represents starch.

**O'Sullivan's Method.**—This method (*J. Chem. Soc., Trans.*, 1884, 45, 1) is slow but gives accurate results with grain. According to Davis and Daish (*J. Agric. Sci.*, 1914, 6, 152) it may, owing to loss of dextrin, yield results 15 to 20% low when applied to leaf material or plant tissues in general.

The finely divided grain is extracted with ether to remove fats and with alcohol to separate sugars, and washed with water to remove amylans. The residue is transformed by diastase into

maltose and dextrin, the proportions of which are determined by Fehling's solution and by the polariscope.

A fair sample of the grain is taken and 5.1 grm. weighed roughly and ground to a fine flour in a clean coffee-mill. 5 grm. of the powder is placed in a flask of about 120 c.c. capacity, thoroughly wetted with rectified spirit, and 25 c.c. of ether added. The flask is corked and agitated occasionally, and after a few hours the ether is decanted through a filter and the residue washed by decantation with three or four fresh quantities of ether. To the residue 80 to 90 c.c. of alcohol (sp. gr. 0.90) is added, the mixture being kept at 35° to 38° for a few hours with occasional shaking. The alcoholic solution, when clear, is decanted through the filter used in filtering the ethereal solution, and the residue washed a few times by decantation with alcohol of the strength and at the temperature indicated. The residue in the flask and any little that may have been decanted on to the filter are then treated together with about 500 c.c. of cold water. In about 24 hours the supernatant liquid becomes clear, when it can be gradually decanted through a filter. The solution filters bright, but, in the case of barley and oats, exceedingly slowly at times; the malted grains, as well as wheat, rye, maize, and rice, yield solutions requiring no excessive time to filter. The residue is repeatedly washed with water at 35° to 38°, but this treatment does not completely free barley and oats from  $\alpha$ -amylan, which substance dissolves with the greatest difficulty at this temperature. The residue is then transferred to a 100 c.c. beaker, and the portion adhering to the filter washed off by opening the filter-paper on a glass plate and removing every particle by means of a camel-hair brush, cut short, and a fine-spouted wash-bottle. When the transference is completed, the beaker, which should now contain not more than 40 to 45 c.c. of the liquid, is heated to 100° for a few minutes in the water-bath, care being taken to stir well when the starch is gelatinising to prevent "balling" or unequal gelatinisation. After this the beaker is cooled to about 62°, and 0.025 to 0.035 grm. of diastase,<sup>1</sup> dissolved in a few c.c. of water, added.

<sup>1</sup> The diastase employed is prepared as follows: 2 or 3 kg. of finely ground pale barley-malt are taken and sufficient water added to moisten and slightly cover it. When this mixture has stood 3 or 4 hours, as much of the solution as possible is pressed out by means of a filter-press. If the liquid is not bright it must be filtered. To the clear bright solution rectified spirit is added as long as a flocculent precipitate forms, the addition of the alcohol being discontinued as soon as the supernatant liquid becomes opalescent or milky. The precipitate is washed with alcohol of sp. gr. 0.86 to 0.88, dehydrated with absolute alcohol, pressed between cloth to free it as much as possible from that liquid, and dried *in vacuo* over sulphuric acid until the weight becomes constant.

Prepared in this way, the substance is a white, friable, easily soluble powder, retaining its activity for a considerable time. The preparation usually sold as diastase is useless for this work.

When the liquid is kept at  $62^{\circ}$  to  $63^{\circ}$  for a short time, the starch is completely converted into maltose and dextrin, and a drop of the solution no longer gives a blue colouration with iodine, but it is desirable to continue the treatment for about an hour after the disappearance of the starch, as the solution then filters more readily. The liquid is next heated to boiling for 10 minutes, and filtered, the residue being carefully washed with small quantities of boiling water. The filtrate is cooled and made up to 100 c.c., and the density observed. The maltose is then determined by means of Fehling's solution, and the dextrin deduced from the rotatory power of the solution. The maltose found, divided by 1.055, gives the corresponding weight of starch, which, added to the dextrin found, gives the total number of grams of starch represented by 100 c.c. of the solution.<sup>1</sup> The sum of the dextrin and maltose found directly ought to agree fairly well with the total solid matter estimated from the density of the solution, after making allowance for the weight of diastase employed.

The A. O. A. C. modifies this method as follows:

Extract 3 grm. of the finely powdered substance on a hardened filter with 5 successive portions of 10 c.c. of ether, wash with 150 c.c. of 10% alcohol, and then with a little strong alcohol. Place the residue in a beaker with 50 c.c. of water, immerse the beaker in a boiling water-bath, and stir the contents constantly for 15 minutes or until the whole of the starch is gelatinised; cool to  $55^{\circ}$ ; add from 20 to 40 c.c. of malt extract and maintain at this temperature until a microscopic examination of the residue treated with iodine reveals no starch. Cool and make up directly to 250 c.c.; filter. Place 200 c.c. of the filtrate in a flask with 20 c.c. of 25% hydrochloric acid (sp. gr. 1.125); connect with a reflux condenser and heat in a boiling water-bath for 2.5 hours; nearly neutralise while hot with sodium carbonate, and make up to 500 c.c. Mix the solution well, pour through a dry filter, and determine the dextrose in an aliquot part. Convert the dextrose into starch by multiplication by the factor 0.9. To prepare the malt-extract required, digest 10 grm. of fresh,

<sup>1</sup> In very accurate experiments it may be well to estimate the  $\alpha$ -amylan present in the solution. For this purpose, 75 c.c. of the above solution should be evaporated to about 30 c.c., cooled, and 60 c.c. of rectified spirit added. A few drops of hydrochloric acid are then added, and the opalescent liquid stirred, when a flocculent precipitate will probably be produced. This is allowed to subside, and the clear supernatant liquid is decanted off. The residue is then washed with alcohol of sp. gr. 0.85, dehydrated by treatment with strong alcohol, and collected on a tared filter. It is then dried *in vacuo* over sulphuric acid, and afterwards in dry air at  $100^{\circ}$ , being subsequently weighed.

finely ground malt for 2 or 3 hours at the ordinary temperature with 200 c.c. of water and filter. Ascertain the amount of dextrose in a given quantity of the filtrate after boiling with acid, etc., as in the starch determination, and make the proper correction.

**Guinness Research Laboratory Methods.**—The various methods proposed for estimating starch in cereals have been examined by H. T. Brown (*Trans. Guinness Research Lab.*, 1903, **1**, 79), who describes a modified form of Soxhlet extraction apparatus designed to facilitate the preliminary removal of the amylans and finds that satisfactory results are given by two methods. The first of these consists in extracting the grain (barley) first with ether and then with alcohol of sp. gr. 0.900, boiling the residue in a flask to expel the alcohol and gelatinise the starch, converting the latter by means of malt-extract and fermenting with yeast, without destruction of the diastase. A check experiment is made with yeast, malt-extract and water, and the amount of alcohol obtained on distilling this liquid applied as a correction to that given in the main experiment; 92 parts of alcohol correspond with 153.9 parts of starch. This method gives accurate results, but complete fermentation occupies some days.

The following method is, however, equally accurate and admits of the estimation of starch in barley in about 5 hours or in malt in 10 to 11 hours. The grain is ground very finely, best in a Maercker mill, and about 5 gm. of it weighed out in a paper thimble and placed in the special extraction apparatus, the moisture being determined on another portion of the ground material. 80 c.c. of alcohol of sp. gr. 0.920 are introduced into the copper flask of the apparatus and the heating adjusted so that the grain is washed with alcohol of sp. gr. 0.900 for 3 hours (this with barley; malt requires 9 hours), when the reducing compounds and alcohol-soluble nitrogen compounds are completely removed; frothing during this extraction may be prevented by addition to the copper flask of about 0.5 gm. of paraffin wax of high melting point. The contents of the thimble are transferred to a beaker containing about 100 c.c. of water and the whole thoroughly boiled, cooled to 57°, mixed with 10 c.c. of an active malt-extract prepared from a malt having a diastatic power of 80 Lintner, and left for 60 minutes. The solution is then boiled and filtered into a 200 c.c. flask, the residue being well washed and the filtrate made up to volume when cool. The copper reduction of 20 c.c. of the solution is determined under the standard conditions

laid down by Brown, Morris and Millar (*J. Chem. Soc., Trans.*, 1897, 71, 94), the maltose being calculated from the copper reduced, corrected for the reduction due to the malt-extract. 84.4 parts of maltose correspond with 100 parts of starch. This holds, however, only when the conversion of the starch is effected by means of a malt-extract prepared from a malt having the diastatic power 80 on the Lintner scale. Ling (*J. Inst. Brewing*, 1922, 28, 838) has investigated the manner in which the yield of maltose varies with the diastatic power of the malt employed and has obtained results which allow of the use of malts of widely varying diastatic powers.

**C. J. Lintner's Polarimetric Method.**—This method for estimating the starch content of barley (*Zeitsch. ges. Brauw.*, 1907, 30, 109) is carried out as follows:

5 grm. of the very finely powdered cereal is triturated in a mortar with 20 c.c. of water until no lumps remain; 40 c.c. of concentrated hydrochloric acid is then added and the mixture left for 30 minutes, the pale yellow paste becoming darker and more fluid. The liquid is then washed into a measuring flask of 200 c.c. capacity by means of hydrochloric acid of sp. gr. 1.125, 10 c.c. of 4% solution of phosphotungstic acid are added to precipitate the proteins, and the volume is made up to 200 c.c. with the diluted hydrochloric acid. The liquid is shaken and filtered and the clear filtrate examined by the polarimeter in a 2 dm. tube. The concentration of the soluble starch is calculated on the basis of  $[\alpha]_D = 200.3^\circ$  for barley starch dissolved in hydrochloric acid at  $20^\circ$ . Provided the liquid be not allowed to remain longer than 2 hours before polarising, no decrease in the rotatory power need be feared. The method gives results 4 to 6% lower than the acid inversion process, owing to the pentosans, etc., being counted as starch in the latter process.

Many methods based on the same principle as that of Lintner have been proposed, and Thorne and Jeffers (*Proc. 7th. Int. Congr. of Applied Chem.*, London, 1909, Sect. 6, A, 14) have drawn attention to the main sources of error in such methods. These are: risk of too advanced hydrolysis if the acid be too concentrated or the temperature too high, and risk of imperfect extraction or of partial reprecipitation if the acid be too dilute at any period of the operation. They find the following modification of Lintner's method to give the best results: 5 grm. of the finely divided material are triturated with about 20 c.c. of water to a smooth cream, hydrochloric acid of sp. gr.

1.15 being added with continued trituration in portions of about 5 c.c. until the mass first swells up into a jelly and finally breaks down into a viscous liquid. This requires about twice as much acid as the water first used, unless the original material contains much moisture, more acid being then necessary. The mixture is allowed to stand for about 10 minutes, and is then transferred to a 200 c.c. flask into which has been previously measured 10 c.c. of 4% phosphotungstic acid solution and 20 c.c. of hydrochloric acid (sp. gr. 1.15). The mortar is rinsed with dilute hydrochloric acid (sp. gr. 1.1) and the whole made up to 200 c.c. with the acid. After mixing, the contents of the flask are transferred to a wide-necked stoppered bottle and thoroughly shaken (or placed in a centrifugal machine) till a well-defined flocculent precipitate is obtained and the supernatant liquid is fairly bright. The rotation of the filtrate is taken in a 200 mm. tube by means of a Schmidt and Haensch quartz compensating polarimeter. The percentage of starch is calculated from the formula,  $S = \frac{R \times 40}{11.6}$ , in which  $R$  is the reading in Ventzke divisions.

**Ewer's Method.**—According to Lintner's method and other similar ones, the assumption is made that the optical activity of substances, other than starch, passing into solution from cereals is sufficiently small to be negligible. Ewers (*Zeitsch. öffentl. Chem.*, 1908, **14**, 8), however, avers that such is not the case and that a control experiment is always necessary to determine the optical rotation of the soluble matters. His method is as follows: 25 c.c. of glacial acetic acid are run into a 200 c.c. flask without wetting the neck, 5 grm. of the finely ground cereal being then added and the flask closed and well shaken until a uniform mixture is obtained. The stopper and the neck of the flask are then washed down with 20 c.c. of the acetic acid and, after the flask has been left in a boiling water-bath for 10 minutes, 10 c.c. of hydrochloric acid (1:10) are added to the flask and the latter left in the bath for exactly 6 minutes with thorough shaking at 1 minute intervals. The volume is next made up to 180 c.c. with hot water and the solution heated for a further period of 15 minutes in the boiling water bath. The liquid is then cooled, clarified by adding 2.5 c.c. of potassium ferrocyanide solution, made up to the mark with water, filtered and polarised. If it is difficult to obtain a clear filtrate, a few crystals of zinc sulphate are added to improve the filtration. The "blank" is carried out

thus: 5 grm. of the finely ground material is shaken vigorously with 70 c.c. of water at 49° in a 100 c.c. flask and the mixture allowed to digest at the ordinary temperature for 1 hour. 25 c.c. of glacial acetic acid are then added and the digestion continued for a further period of 30 minutes, after which the liquid is cooled, treated with ferrocyanide, diluted to 100 c.c., filtered and polarised; the reading obtained is applied as a correction to that obtained as above. For rice starch  $[\alpha]_D = 186.07^\circ$  and for maize starch  $184.19^\circ$  at 20°; these values are for 5% solutions, but the rotation varies little with the concentration within moderate limits.

**Davis and Daish's Taka-diastase Method.**—One of the most valuable investigations on the estimation of starch is that carried out by Davis and Daish (*J. Agric. Sci.*, 1914, 6, 152), who have devised a new method based on the use of Taka-diastase. The action of this diastase yields solely maltose and dextrose, so that basic lead acetate or other clarifying agent may safely be added to the solution, there being no dextrin to undergo partial precipitation together with the tannins, proteins, etc., it is desired to remove. The method is of particular value for the estimation of starch in leaves and other plant tissues containing relatively large proportions of substances other than starch, but is applicable also to cereal grains, etc.

In the case of living plant tissue, the material is first plunged into boiling 95% alcohol containing 1% by volume of ammonia solution (0.880), the enzymes present being thus destroyed. In order to expel soluble carbohydrates the material is extracted thoroughly (for 18 to 24 hours) with boiling 80% alcohol in a Soxhlet extractor, and is then pressed to remove alcohol and dried in a steam oven for 18 hours and ground. About 10 grm. is then dried until the weight is constant in a vacuum over phosphoric oxide at 100° to 110°. In many cases these preliminary operations may be abbreviated considerably.

Horton (*J. Agric. Sci.*, 1921, 11, 240) has shown that in using this method it is necessary to make tests with pure starch in the case of each preparation of the enzyme before and during use.

The dry material (free from sugars and, if necessary, previously extracted with water to remove gums, amylans, etc., see page 536) is gelatinised with 200 c.c. of water in a 250 c.c. beaker flask heated for 30 minutes in a water-bath at 100°. The solution is cooled to 38°,

0.1 grm. of Taka-diastase is added,<sup>1</sup> together with 2 c.c. of toluene, and the mixture left 24 hours in order that the conversion may take place; it is then heated in a boiling water-bath to destroy the diastase and the clear solution above the residual material is filtered through a fluted filter paper into a 500 c.c. measuring flask; the residue is thoroughly washed several times by decantation, the washings being passed through the filter paper until the volume of liquid in the flask amounts to about 475 c.c. The necessary quantity of basic lead acetate is then added to precipitate the tannins, etc., present in the solution. The amount required varies considerably with different leaves or other material, generally ranging from 5 c.c. to 25 c.c. A large excess of lead should be avoided and tests should be made after each small addition of lead acetate in order to ascertain when the precipitation is complete. When this is the case the solution is made up to 500 c.c. at 15°, and filtered; 100 c.c. of the filtrate is placed in a 110 c.c. measuring flask, the slight excess of lead precipitated by adding solid sodium carbonate and the volume adjusted to 110 c.c. at 15°. 50 c.c. of the filtrate from the lead carbonate is used for the reduction and another portion polarised in a 400 mm. tube. The following example shows the method of calculation:

Weight of extracted leaf material ( <i>Tropaeolum majus</i> ) after drying in steam oven	= 10.4122 grm.
Weight of leaf material dried <i>in vacuo</i> at 100°	= 9.4059 grm.
CuO from 50 c.c. of the final 110 c.c.	= 0.4492 grm.
Polarisation of this solution in 400 mm. tube at 20°	= 1.995°

If  $x$  = grams dextrose in 50 c.c. of this solution,  
and  $y$  = grams maltose in 50 c.c. of this solution,

we have, using the values of CuO corresponding with 1 grm. of dextrose and maltose for the weight 0.4492 CuO in the tables of Brown, Morris and Millar:

$$2.369x + 1.362y = 0.4492 \quad (1)$$

For the 400 mm. tube, employing the values  $[\alpha]_D^{20} = 137.6^\circ$  and  $[\alpha]_D^{20} = 52.7^\circ$  for maltose and dextrose we have also

$$4.216x + 11.008y = 1.995 \quad (2)$$

Solving equations 1 and 2 for  $x$  and  $y$

$$x = 0.1095 \text{ grm. dextrose in 50 c.c.}$$

$$y = 0.1394 \text{ grm. maltose in 50 c.c.}$$

$$\text{Total dextrose in 500 c.c. original solution} = 0.1095 \times \frac{110}{50} \times 500 = 1.2045 \text{ grm.}$$

$$\text{Total maltose in 500 c.c. original solution} = 0.1394 \times \frac{110}{50} \times \frac{500}{100} = 1.5334 \text{ grm.}$$

$$\text{Starch corresponding with dextrose} = 0.90 \times 1.2045 = 1.0840 \text{ grm.}$$

$$\text{Starch corresponding with maltose} = 1.5334 \div 1.055 = 1.4535 \text{ grm.}$$

$$\text{Total starch} = 2.5375 \text{ grm.}$$

∴ per cent. of starch in vacuum dried extracted leaf material

$$2.5375 \times \frac{100}{9.4059} = 26.97$$

<sup>1</sup> The commercial preparation of Messrs. Parke, Davis & Co. may be used.

### Precautions Necessary in Taking Samples for Analysis

If the dried, ground plant material is bottled before analysis, it is absolutely necessary when each sample is taken for the analysis *to turn out the whole of the material on to a sheet of paper and mix it thoroughly before sampling*. If this precaution is not observed and successive samples are taken directly from the bottle, it is frequently found that the proportion of starch present in the material increases towards the bottom of the bottle. This is no doubt due to the fact that the heavier starch grains, set free from the tissue by grinding, sink to the bottom of the bottle, whilst the lighter fibrous material rises to the top. This is well shown by the following successive analyses made with potato leaves (previously freed from sugars by extraction):

Sample from top of bottle, starch	=	7.54% on vacuum dried matter.
Sample from middle of bottle	=	9.19% on vacuum dried matter.
Sample from middle of bottle	=	9.23% on vacuum dried matter.
Sample from bottom of bottle	=	12.29% on vacuum dried matter.

When, however, the sampling is carried out in the way described above the agreement between different individual determinations is as satisfactory as could be expected in work of this class.

One of the principal difficulties in estimating starch in plant material is due to the presence of gummy substances, tannins, proteins, etc., which pass into solution during the hydrolysis and exercise an effect on the rotatory and reducing powers of the solution. These substances are very largely removed by the use of basic lead acetate, but sufficient impurity remains, even after this treatment, to falsify the analyses in some cases. Thus in the case of mangold leaf a lævorotatory gum is present, which gives an error of nearly 10% on the rotation actually measured.

In working with plant material it is generally possible to extract the disturbing gummy substance prior to the starch conversion by preliminary treatment with water.<sup>1</sup> Thus, in the case of the mangold leaf, by adding 200 c.c. of water and 5 c.c. of toluene to the leaf material and extracting for 24 hours at 38°, decanting and washing with a little water and subsequently converting with Taka-diastase, in the ordinary way, a solution is finally obtained (after the usual treatment with basic lead and sodium carbonates) which, in a 400 mm. tube, shows a lævorotation of not more than 0.01°. It

<sup>1</sup> Compare Brown and Millar, *Trans. Guinness Research Lab.*, 1903, 1, 79.

is noteworthy that the preliminary treatment with water fails to remove the greater part of the material precipitable by basic lead acetate, so that this treatment is necessary even after the preliminary extraction with water.

In the case of plant material from which gummy matter is extracted with extreme difficulty, it would probably be sufficient to introduce a correction for any active substances present by carrying out a control experiment or "blank" in which the diastase is omitted but the material is otherwise treated exactly as in the actual estimation of starch.

*Other Methods for Estimating Starch.*—Of the many other suggested methods, some of which have the advantage of rapidity and may be applied under certain conditions, the following deserve brief mention. Lindet (*Bull. Soc. Chim.*, 1896, [iii], **15**, 1163) treats the ground cereal with pepsin to remove the protein enclosing the starch granules, a small proportion of hydrochloric acid being added at the same time to prevent the action of the amylolytic enzymes. The starch is subsequently separated and weighed. Kaiser's method (*Chem. Zeit.*, 1902, **26**, 180) consists in precipitating starch paste quantitatively by means of iodine in presence of sodium acetate, treating the precipitate with alcoholic potassium hydroxide to reconvert it into starch and weighing the latter.

Baumann and Grossfeld (*Zeitsch. Unters. Nahr.-Genussm.*, 1917, **33**, 97) utilise the complete precipitation of starch by lead tannate formed in the solution; from the diminution in rotation resulting from the precipitation and removal of the starch, the percentage of the latter is calculated, the average value of the specific rotation of various starches being found to be  $183.7^\circ$ . Von Fellenberg's method (*Mitt. Lebensmittelunters. Hyg.*, 1916, **7**, 369) is based on the solubility of starch in 50% calcium chloride solution, subsequent precipitation by means of iodine, decomposition of the precipitate with alcohol, and direct weighing of the starch. Mannich and Lenz (*Zeitsch. Unters. Nahr.-Genussm.*, 1920, **40**, 1) also make use of the solubility of starch in concentrated calcium chloride solution, but by addition of a small proportion of acetic acid obtain a clear solution suitable for direct polarimetric observation.

For estimating starch in flour and other starch products, Quisumbing (*Philippine J. Sci.*, 1920, **16**, 581) treats the gelatinised material with saliva until no blue colouration is given with iodine solution

and then he boils the resultant liquid with dilute sulphuric acid; the neutralised solution is heated with sodium carbonate and permanganate, the excess of the latter being estimated by means of oxalic acid.

**Commercial Starches.**—These are usually obtained from wheat, rice, maize or potatoes. In England hardly any starch is now made from wheat. Characteristic of wheat starch is the coherence of the granules due to the small admixture of gluten. A rough estimation of the starch in wheat flour may be effected by washing a weighed quantity over a muslin sieve in a stream of water. The gluten remains and the water containing the starch is allowed to stand until the starch has settled, when it is collected, dried at  $110^{\circ}$  and weighed.

The ash of starch is trifling in amount, and its estimation serves to detect any mineral additions.

The moisture may be determined by drying in a vacuum over sulphuric acid or in a current of dry air at  $100^{\circ}$ . For estimating approximately the water in potato starch, Saare's method is more convenient. It consists in placing 100 grm. of the sample in a 250 c.c. flask, filling the flask to the mark with water at  $17.5^{\circ}$ , and observing the weight of the contents. There is no occasion to employ the large quantities of starch and water recommended by Saare. He gives a table (*J. Soc. Chem. Ind.*, 1884, 3, 527) by which the proportion of water is directly shown, but the following rule may be employed instead: from the weight of the starch and water contained in the bottle subtract 250, and divide the remainder by 0.3987, when the quotient will be the percentage of starch in the sample. This instruction applies to the quantities of starch and water prescribed by Saare, but the following is a more general expression of the rule:

$$\frac{\text{Contents of bottle in grams minus capacity of bottle in c.c.}}{0.3987} = \left\{ \begin{array}{l} \text{grams of anhydrous starch in} \\ \text{weight of sample taken.} \end{array} \right.$$

The method gives values within 0.5% and an estimation can be made in 30 minutes. J. F. Hoffmann and J. H. Schulze (*Woch. für Brauerei*, 1903, 20, 217) heat the starchy matter to drive out the water, which is condensed, collected and measured. 50 grm. of starch is immersed in 400 c.c. of oil of turpentine and 10 c.c. of toluene in a boiling vessel and heated first at  $50^{\circ}$ , then at  $140^{\circ}$  and finally at  $155^{\circ}$ , for 5 minutes in each case. The water formed is collected and measured, a correction of 0.2 c.c. added and the whole multiplied by 2 to give the percentage of water in the starch.

For technical purposes it is sometimes desired to estimate the proportion of starch existing in potatoes. This may be done in a rough and ready manner by ascertaining the sp. gr. of the tubers. The unpeeled potatoes, freed from dirt, are placed in a solution of salt, which is then diluted with water till some of the individual tubers sink, while others just float. The density of the saline solution, as ascertained by a hydrometer, is then approximately equal to the average sp. gr. of the potatoes. Another method consists in taking 5 kg. of the potatoes and weighing them in water. The loss of weight in water divided into the original weight in air gives the sp. gr. Tables have been compiled for ascertaining the percentage of starch from the sp. gr. of the potatoes. If such tables are not available, results very approximately exact may be deduced from Rüdiger's formula,  $S = \frac{U - 90}{20}$ , where  $S$  represents the percentage of starch in the potatoes and  $U$  the under-water weight in grams of 5 kg. of dry, or 5.05 kg. of wet, washed potatoes.

The sp. gr. ranges from 1.08 to 1.15, the heaviest potatoes containing most starch and most dry matter. The tables most used are those of Behrend, Maercker and Morgen (*Zeitsch. Spiritusind.*, 1879, 361), which give results accurate within about 1%. An approximate estimation may be based on the following: a potato containing 19.9% of dry matter has the sp. gr. 1.081; an increase of 0.001 in the sp. gr. corresponds with 0.214% of dry matter. The percentage of dry matter less 5.75 is equal to the percentage of starch present.

Commercial starches may contain extraneous mineral matters, such as sand, gypsum, chalk, baryta, alumina, etc., as well as foreign organic substances, for example, bran, potato residues, fungi, bag fibres, etc. Added mineral matter may be detected by shaking a few grams of the powdered sample in a test-tube with chloroform and then allowing to stand; the starch floats, whilst the mineral matter gradually sinks and may be analysed separately. The nature of the inorganic adulterants may be determined also by analysis of the ash of the starch. Extraneous organic materials may be detected by examining the smoothed surface of the starch either with the naked eye or, better, with the help of a lens; such impurities then appear as dark or coloured spots on the white surface, and the number of spots

per square inch affords a rough indication of the extent of the contamination.

Starches are often graded according to the stiffness of the pastes they yield under standard conditions when boiled with water and cooled. Ermen (*J. Soc. Chem. Ind.*, 1907, **26**, 501) makes solutions of starch in the cold with the help of sodium hydroxide and determines their viscosities in a Redwood viscometer. The weighed sample of starch is shaken with 230 c.c. of cold water and 15 c.c. of a 10% solution of sodium hydroxide with the addition of enough water to bring the whole up to 250 c.c., the shaking being continued until the solution begins to thicken. It is allowed to stand until the next morning before measurement of the viscosity. When close attention is paid to constancy of procedure, the method is claimed to give concordant results with starches of similar origin, whilst different starches and different brands of the same starch are easily differentiated.

Starch to be used for dressing textiles is usually subjected to suitable tests in the works or in special laboratories.

### DEXTRIN. AMYLIN

What actually constitutes a dextrin is not easy to define, the name having been given to many different substances or mixtures of substances prepared in various ways from starch and starch-containing materials, and intermediate in properties between starch and maltose. Such products are obtained by:

Maintenance of starch or flour at a temperature ranging from 210° to 280° until it acquires a yellow or brownish colour. The change is greatly facilitated by moistening the starch with dilute nitric or other acid, and then slowly drying the paste and heating it for some time to about 110° to 150°. In such cases lighter coloured dextrins are obtained than when no acid is present during the heating.

Boiling of starch with dilute sulphuric acid until the cooled liquid no longer gives a colouration with iodine solution.

Treatment of gelatinised starch with warm water and a small quantity of malt-extract.

The first process is employed for the manufacture of solid dextrin, which is known in commerce by the name of British gum, gommeline, starch-gum, etc. The other processes result in the simultaneous

formation of maltose, as described elsewhere. The second is used for the preparation of commercial glucose, and the last reaction takes place when malt is mashed for the manufacture of beer.

Several, and possibly many, varieties of dextrin exist, all being apparently formed by the breaking down of the highly complex starch molecule. According to the degree of degradation which the starch has suffered, the properties of the resulting dextrins, such as the magnitudes of the optical activity and of the copper reducing power, the colour given with iodine solution, the diffusibility through animal and vegetable membranes, etc., resemble more nearly those of the original starch or those of maltose. They do not form phenylosazones. Some dextrins are totally unfermentable by all yeasts, but certain of them may be fermented, partially at least, by specific yeasts. There is no ready method of distinguishing the different varieties with certainty, except that those of one kind, or possibly class, give a reddish-brown colour with solution of iodine, whilst those of the other kind or class produce no such colouration. Erythrodextrin, which gives the brown colour with iodine, is an intermediate product in the formation of achroo-dextrin from starch.

The best method of applying the iodine reaction is to divide a very weak solution of iodine in potassium iodide into two parts, and place the slightly yellow liquid in adjacent test-tubes or glass cylinders. On adding the solution to be tested to one, and an equal volume of water to the other tube, any brownish colouration will be readily observed. In presence of starch, the blue is apt to obscure the brown tint produced by erythrodextrin; this difficulty may be avoided to some extent by employing the iodine solution somewhat in excess, so as to get a full development of the brown colour.

Pure dextrins are white or almost white amorphous solids and are tasteless, odourless, non-volatile and highly deliquescent. They are insoluble in alcohol, but readily soluble in water giving, when concentrated, syrupy liquids. The value of  $[\alpha]_D$  varies widely but is mostly about  $200^\circ$ .

Cold concentrated sulphuric acid dissolves dry dextrins without colour, but charring takes place on warming. When boiled with dilute acids, dextrins yield maltose and ultimately dextrose. Hot nitric acid of sp. gr. 1.35 converts dextrins in part into oxalic acid, whereas the natural gums yield mucic acid under similar conditions.

Dextrins are distinguished from starch by their solubility in cold water; from soluble starch by yielding no blue with iodine when tested as described on page 527, and no precipitate with baryta water; from maltose and dextrose by not reducing Fehling's solution; from starch, soluble starch, gelatin and egg-albumin by not yielding a precipitate with tannin; from albumin by not being coagulated by heat or mineral acid.

Dextrins are separated from starch and cellulose by solution in cold water; coagulable proteins may then be separated by raising the faintly acid solution to boiling. An ammoniacal solution of lead acetate added to the cold and dilute liquid is stated to precipitate the dextrin, leaving the sugar in solution; the precipitate may be dried at  $100^{\circ}$ , and is said to have the formula  $\text{PbO}, \text{C}_6\text{H}_{10}\text{O}_6$ . Another method consists in precipitating the dextrin by means of a large proportion of alcohol, washing the precipitate with rectified spirit, and drying it at  $110^{\circ}$ . After weighing, the dextrin should be ignited, and the resultant ash deducted from the total weight obtained.

The proportion of dextrin present in a solution containing also maltose and dextrose may be determined by observing the rotatory action of the liquid, together with its sp. gr. and reducing action on Fehling's solution.

**Commercial Dextrins.**—Most dextrins which are almost white or yellow are made with the help of acids, brown dextrins being obtained by roasting alone. The highest qualities, prepared from good potato starch, are lustrous, those with a dull surface being usually obtained from wheat or maize starch. The odour, which is accentuated by moistening the sample with water, should give no indication of mould; acidity or the presence of saccharine matter is sometimes revealed by the taste.

Dextrin made by the action of heat alone may retain the structure of the original starch sufficiently for microscopic examination (in oil or glycerol) to show its origin.

The manner in which any particular dextrin should be examined depends on the use to be made of it. Thus, determination of the acidity, ash, matters insoluble in hot or cold water, starch, etc., may give useful indications in some cases. A knowledge of the viscosity of a solution of the dextrin, measured at a certain temperature and compared with that of a solution of a standard dextrin

under the same conditions, is sometimes of value. Chlorine, occasionally used to bleach either the starch or the resultant dextrin, may be detected in the ordinary way.

For dressing textiles and for employment in dyeing, dextrins capable of forming solutions which remain pasty for a long time are desired; here, too, comparison with standard specimens is advisable. Further, for such dextrins Bolis (*Ind. Tessile e Tintoria*, 1903, 117) has devised the following tests: (1) a little perfectly white, pure wool is treated with the following mixture and then steamed, washed and dried:

Ammoniacal cochineal solution containing 30 grm. of cochineal per litre.....	1 litre
Powdered alum.....	24 grm.
Oxalic acid.....	10 grm.
Dextrin.....	375 grm.

The wool should show a good pink tint quite free from yellow.

(2) Cotton is treated with the following mixture:

Aluminium acetate prepared with 36.5 grm. of alum per litre of water.....	1 32 litres
Water.....	15.32 litres
Dextrin.....	150.00 grm.

and is then dyed with alizarin, when it should exhibit a bright pink tint.

"British gum" is a white, yellowish or pale brown powder, which consists largely of erythrodextrin, its aqueous solution being coloured brown with iodine, unless this reaction is obscured by the blue produced by a considerable proportion of soluble *starch*; for most purposes such admixture is unobjectionable, provided that it does not exceed 12 or 15%. *Unaltered starch* may be recognised microscopically and by its insolubility in cold water. Reducing *sugars* (maltose) are nearly always present in commercial dextrin, and may be detected and estimated by means of Fehling's solution.

Many mixtures of starch and dextrin are employed as thickening agents in calico-printing, etc. Liquid or semi-liquid pastes of the nature of "Gloy" are prepared by the restricted hydrolysis of starch, mostly wheat starch, in presence of magnesium chloride, zinc chloride, etc.

Dextrin syrups are largely employed by confectioners. Their examination is described under "glucose."

The method of distinguishing commercial dextrin from gum arabic is described on page 560.

Useful information concerning the examination of commercial dextrans is given by Lamb and Harvey (*J. Soc. Dyers and Col.*, 1918, 34, 10), and by Babington, Tingle and Watson (*J. Soc. Chem. Ind.*, 1918, 37, 257T).

### CELLULOSE,<sup>1</sup> C<sub>6</sub>H<sub>10</sub>O<sub>5</sub>

Cellulose constitutes the essential part of the solid frame-work or cellular tissue of plants and is a characteristic product of the vegetable kingdom. Tunicin, a compound resembling cotton cellulose in many ways, is obtained from the outer coating of *Ascidia* and other invertebrate species.

Cellulose occurs nearly pure in cotton, linen and the pith of certain plants. Still purer forms are Swedish filter-paper,<sup>2</sup> linen rags and cotton-wool. Cellulose is more stable than starch. It is insoluble in water or any of the common solvents. Air-dry cellulose contains from 6 to 12% of water, which is readily driven off at 100°, but is reabsorbed on exposure to the atmosphere, and is termed "moisture of condition." This moisture is of great importance in the processes of spinning and finishing fibres and also in the buying and selling of fibrous products.

Cellulose hydrates give an indigo-blue colouration with iodine in aqueous solution. They do not, however, differ in their essential properties from cellulose.

The water of hydration of a cellulose may be determined as the difference between the hygroscopic moisture lost at 100° to 105°, and that driven off at the temperature of boiling toluene. The sample is boiled with toluene and the water which distils over absorbed by calcium chloride and weighed after expulsion of the hydrocarbon.

To determine the degree of hydration of a cellulose, the sample is stained blue with zinc chloride-iodine reagent and the rapidity with which the colour is removed by water noted. Highly hydrated celluloses retain the colour for a considerable time.

<sup>1</sup> For full information on the subject of cellulose the work of Cross and Bevan on *Cellulose* should be consulted, also their "*Researches on Cellulose*," 1895-1900, 1900-1905, 1905-1910, 1910-1920 (Cross and Dorée).

<sup>2</sup> For special purposes bleached celluloses are tested by treatment with sodium hydroxide solution, being resolved into:

α-cellulose, resistant to sodium hydroxide,

β-cellulose, dissolved by sodium hydroxide and reprecipitated by acetic acid, and

γ-cellulose, remaining soluble in the filtrate from β-cellulose. Some Swedish filter-papers contain 2 to 5% of β- and γ-celluloses. (Cross).

**Solvents of Cellulose.**—Cellulose is hydrated and dissolved by: (1) aqueous zinc chloride: 4 to 5 parts of zinc chloride are dissolved in 5 to 7 parts of water and 1 part of cotton cellulose stirred in till evenly moistened, the solution being heated in a porcelain dish over the water-bath. It is stirred from time to time and water added to replace that which evaporates. The solution forms a precipitate of cellulose hydrate containing zinc salt when poured into water or alcohol; digestion with dilute hydrochloric acid removes the zinc salt. This solution is employed for making cellulose threads which are carbonised for use in the incandescent electric lamp. Zinc chloride dissolved in twice its weight of 40% aqueous hydrochloric acid dissolves cellulose rapidly in the cold, but the cellulose subsequently undergoes gradual hydrolysis.

2. Ammoniacal cupric oxide (Schweitzer's reagent) contains 10 to 15% of ammonium hydroxide and some 3% of CuO. It is prepared either by adding ammonium chloride and an excess of sodium hydroxide to a solution of a cupric salt and redissolving the well-washed blue precipitate in ammonia solution (sp. gr. 0.92), or by immersing copper turnings in strong ammonia solution in a cylinder and bubbling air or oxygen through the liquid for about 6 hours. On treatment with the cuprammonium solution, cellulose becomes gelatinous and on agitation gradually dissolves forming a viscid solution which may be filtered after dilution with water.

When the filtrate is neutralised with hydrochloric acid the cellulose is separated in a flocculent state resembling aluminium hydroxide, and when dried forms a brittle, greyish, horn-like mass. Carbon dioxide also precipitates the solution, as do sugar, salt and even copious dilution with water.

The solution of cellulose in Schweitzer's reagent is decomposed by dialysis. It is laevorotatory, a 1% solution showing a specific rotation of  $-20^\circ$  for the light transmitted. The optical activity is not strictly proportional to the cellulose dissolved, increasing somewhat more slowly than the concentration of the solution.

Fabrics passed through a bath of the reagent are coated on the surface with the film of gelatinised cellulose and rendered compact so that the fabric becomes waterproof. The cellulose retains the copper hydroxide which acts as a preservative.

Other solvents of cellulose are: (1) ethylenediamino-cupric solutions, which act more powerfully than ammoniacal cupric oxide

solutions, and (2) concentrated solutions of various thiocyanates at temperatures of  $120^{\circ}$  to  $140^{\circ}$ .

Cellulose and its decomposition products are soluble in concentrated hydrogen peroxide solution (up to 60%), giving solutions from which precipitants, such as water, throw out the cellulose in chemically modified condition (Bamberger and Nussbaum, *Monatsh.*, 1919, 40, 411).

The colloidal characteristics of cellulose, which are rendered evident by all reactions resulting in soluble products, are discussed by C. F. Cross in *Lectures on Cellulose*, published by The Institute of Chemistry, 1912.

When subjected to prolonged boiling with dilute acids, cellulose is converted into hydrocellulose, which differs from cellulose in containing free carbonyl groups and in the greater reactivity of its hydroxyl groups. Cold concentrated sulphuric acid rapidly attacks and dissolves cellulose, with the formation of dextrin-like substances. If this solution is diluted and boiled, dextrose is formed as the chief product of hydrolysis. Hot concentrated sulphuric acid at once chars cellulose. On treatment with sulphuric acid diluted with half its volume of water (sp. gr. 1.5 to 1.6) cellulose is gelatinised and converted into a substance termed amyloid, which is coloured blue by iodine. Paper placed in an acid of this strength for a short time and then transferred to water has a tough coating of amyloid fixed on its surface and constitutes parchment paper.

By treatment with cold nitric acid of sp. gr. 1.42 cellulose is remarkably toughened, without losing its fibrous structure or becoming nitrated. With stronger acid, cellulose is converted into nitrated products which are described elsewhere; the limit of esterification is reached when three ( $\text{O.NO}_2$ ) groups are introduced per  $\text{C}_6\text{H}_{10}\text{O}_6$ .

Nitric acid (sp. gr. 1.1 to 1.3) oxidises cellulose to oxycellulose; dilute chromic acid has a similar effect. Hypochlorites in dilute solution (1%) have only a very slight action on cellulose proper. Permanganates in neutral solution also attack cellulose but slowly, whilst stronger solutions oxidise and disintegrate the fibre substance, an oxycellulose resulting. The joint action of hypochlorite solution and carbonic acid rapidly produces oxycellulose, which acquires the property of selective attraction for certain colouring matters—notably the basic coal-tar dyes. Oxycelluloses in overbleached cloth

may thus be easily detected by immersion of the fabric in a dilute solution of methylene blue.

Cellulose is very resistant to dilute alkali solutions, even at high temperatures. By drastic treatment with 1 to 2% sodium hydroxide solution cellulose fibres are freed from non-cellulose constituents, which become saponified. Cold solutions containing above 13% of sodium hydroxide cause a remarkable change in the structure of the fibre which, seen in the mass, exhibits a shrinkage in length and width with an increase in thickness. The compound of cellulose and alkali formed is decomposed when washed with water, the cellulose reappearing as the hydrate  $C_{12}H_{20}O_{10}$ ; this is known as mercerised cellulose.

This alkali-cellulose reacts with carbon disulphide to form an alkali-cellulosexanthate perfectly soluble in water, giving a very viscous solution which is precipitated in the form of a gelatinous hydrate by various neutral dehydrating liquids or solutions. To prepare it, cellulose is treated with excess of 15% sodium hydroxide solution and after standing for some time separated from the liquid, squeezed to remove excess and mixed with 40 to 100 parts of carbon disulphide. After some hours, the yellowish mass is covered with water and subsequently stirred with more water, when solution occurs.

Cellulose is regenerated from this solution on standing some days or on heating at  $80^{\circ}$  to  $90^{\circ}$ . It has 3 to 4% more moisture and corresponds with the formula  $4C_6H_{10}O_5 \cdot H_2O$ . In general it is far more reactive than the original cellulose.

If cotton-wool or filter-paper is heated at  $180^{\circ}$  for several hours with about 6 or 8 parts of acetic anhydride, it is entirely dissolved and converted into a triacetate,  $C_6H_7(C_2H_3O)_3O_5$ , which may be separated by pouring the syrup into water; it is a white powder, optically inactive, soluble in strong acetic or sulphuric acid, and very readily converted into cellulose and potassium acetate by boiling with dilute potassium hydroxide solution. Hydrolysed derivatives of cellulose undergo more advanced acetylation. A fibrous monobenzoate and an amorphous dibenzoate are known, but no higher formate than the monoformate is known definitely (Cross and Bevan, *J. Chem. Soc., Trans.*, 1911, **99**, 1450).

The action of sulphuric acid on cellulose under suitable conditions yields disulphuric esters, but at the same time the cellulose aggregate

undergoes disintegration. The complex series of products known as hydrocelluloses and oxycelluloses, obtained by the action of aqueous acids and oxidising agents, differ only fractionally in composition from  $nC_6H_{10}O_5$  (Cross, *Lectures on Cellulose*, Institute of Chemistry, 1912).

The estimation of the cupric reducing power is said to afford a useful measure of the free carbonyl groups in cellulose and hence of the chemical condition of the sample. Schwalbe (*Ber.*, 1907, 40, 1347) determines this in the following manner:

Two portions, of about 3 grm. each, of the cellulose are weighed out; one serves for the estimation of the absolute dry substance, whilst the other is reduced to a state of fine division, without drying by heat, and is mixed with 200 c.c. of water and 100 c.c. of Fehling's solution, the liquid being boiled for 15 minutes under a reflux condenser, with frequent agitation. The solution is then filtered hot and the residue, containing the cuprous oxide, is dissolved in nitric acid and the amount of copper determined, preferably electrolytically. The "copper value" represents the percentage of metallic copper calculated on the dry cellulose. Cotton wool has the value about 1.7, bleached sulphite wood pulp about 3.9, overbleached sulphite wood pulp 19.3.

In a second paper (*Ber.*, 1907, 40, 4523) Schwalbe applies the test to artificial silks. Viscose and Pauly silks, both made by alkali processes, have low copper values, namely, about 0.8. Chardonnet silk, being made by an acid process, has the value 3.1.

Hydrocelluloses show values of 2 to 8.8 according to the degree of hydrolysis. Oxycelluloses have much higher copper values—7.6 to 35.

Plant celluloses may be classified in three groups (Cross and Bevan):

1. Those of maximum resistance to hydrolysis and containing no carbonyl groups—normal cellulose.
2. Those of less resistance to hydrolysis and containing active carbonyl groups—the oxycelluloses.
3. Those which are easily hydrolysed by acid or in some cases by enzymes to form simple carbohydrates and which are more or less soluble in alkaline solutions—the pseudo- or hemi-celluloses.

The results obtained by Schwalbe and Becker (*J. prakt. Chem.*, 1919, [iii], 100, 19) by boiling various hydro- and oxy-celluloses with solutions of calcium, strontium and barium hydroxides or magnesium

oxide indicate that such products are merely mixtures of pure cellulose and its degradation products. For instance, bleaching powder oxycellulose contains about 60% of pure cellulose and 40% of apparently oxidised degradation products. A variety of cellulose practically devoid of reducing properties is obtainable by the action of boiling milk of lime on all sulphite celluloses, hydro- and oxycelluloses.

To establish the nature of a cellulose, it is generally sufficient to determine the ultimate composition, resistance to alkaline hydrolysis, behaviour with solvents, reaction with sulphuric acid (solution without blackening) and with a nitrating mixture (nitric and sulphuric acids).

Materials composed of normal and resistant celluloses only are quite inert and have lasting properties. Those containing oxidised and oxycelluloses, also lignocelluloses, are liable to discolouration and are far more perishable.

*Compound Celluloses.*—In these substances—the lignocelluloses, cutocelluloses (adipocelluloses), and pectocelluloses—the nature of the union between the cellulose and the non-cellulose components does not appear to be the same in all cases. With the cutocelluloses such union is almost certainly chemical in character, but with the lignocelluloses the union exhibits rather the quality of a mutual interpenetration or absorption, although the combination is as resistant to reaction as one of chemical type.

The **lignocelluloses**, of which the jute fibre is a typical representative, differ markedly from the simple celluloses. They have a higher  $\frac{\text{CH}}{\text{O}}$  ratio, and contain unsaturated groups which combine with chlorine to form quinonoid substances. They contain a furfural-yielding complex, methoxyl groups and an acetic acid residue. These constituents make up the non-cellulose part (often termed lignone) of the fibre; the latter contains also cellulose proper which can be isolated only after chemical decomposition of the non-cellulose.

Lignocelluloses exhibit a number of characteristic reactions. Salts of aniline colour the fibre a deep golden-yellow. Phloroglucinol, dissolved in hydrochloric acid, gives a deep magenta colouration. Iodine is absorbed in large quantity, colouring the fibre a deep brown. The fibre readily combines with chlorine, as is shown by the characteristic magenta colouration developed on subsequent addition of

sodium sulphite. Very characteristic is the reaction with ferric ferricyanide obtained by mixing equivalent proportions of potassium ferricyanide and ferric chloride; the fibre is stained a deep blue and takes up a considerable quantity of pigment.

**Pectocelluloses.**—In these, which contain less carbon and more oxygen than the celluloses proper, the non-cellulose constituent belongs to the pectic substances or pectins, which are colloidal, carbohydrate aggregates of hexosans and pentosans, together with a small proportion of acid groups combined as lactone or ester.

**Pectose** occurs in the utricular tissue of fruits and roots. It is insoluble in water, but is converted into soluble pectin on hydrolysis with dilute acids or alkalies or by the enzyme, *pectase*. In addition to the pectocelluloses proper, typified by flax, esparto, etc., are the *mucocelluloses*; these are decomposed by the action of water, forming the solutions known as mucilages, which are neutral and on ultimate hydrolysis give rise to various hexoses and pentoses.

**The cutocelluloses** are associated with oily and waxy products, which add to their water-resisting property, but are removed by solvents. On decomposition by oxidation and saponification, a large additional quantity of such products is formed.

**Cutose**, or cuticular substance, constitutes the greater part of cork, and also the fine transparent membrane covering the exposed parts of vegetables. It contains a high percentage of carbon ( $C = 68.29$ ;  $H = 8.95$ ), and yields suberic acid,  $C_8H_{14}O_4$ , when oxidised by nitric acid of sp. gr. 1.2. Cutose is insoluble in cold sulphuric acid of sp. gr. 1.78 and in the cuprammonium solution which dissolves cellulose. On the other hand, it dissolves slowly in a hot dilute solution of sodium hydroxide or carbonate, forming a solution from which acids precipitate a yellowish, flocculent substance, fusible below  $100^\circ$ , soluble in alcohol and ether, and having the same composition as cutose. If the alkaline solution is saturated with common salt, a cutose-soap rises to the surface. Cutose appears to be composed of stearocutic acid,  $C_{28}H_{48}O_4$ , with five equivalents of oleocutic acid,  $C_{14}H_{20}O_4$ .

**Purification of Cellulose in the Laboratory.**—(1) The fibre is first subjected to alkaline hydrolysis, *i. e.*, boiling with dilute sodium hydroxide, carbonate or sulphite.

2. It is then exposed to chlorine gas or bromine water or oxidised by means of hypochlorite or permanganate. The use of the last

necessitates subsequent treatment with sulphurous acid to remove the manganese dioxide deposited on the fibre.

3. Finally process 1 is repeated to remove products rendered soluble by process 2.

From *starch* cellulose is best separated by boiling the substance with water containing 1% by volume of sulphuric acid. The liquid is filtered when a drop taken out gives no colouration with iodine solution. In cases in which the use of acid is objectionable, the substance should be boiled with water, and the unfiltered liquid mixed with an equal measure of a cold infusion of malt. The starch will be wholly dissolved by keeping the liquid at a temperature of 60° for a short time. The separation of cellulose from *sugar*, *dextrin* and other substances soluble in water presents no difficulty. Proteins may be separated by treatment with warm water containing 1% of alkali. They may be determined by the methods for estimating nitrogen.

*Estimation of Cellulose.*—No method is known for precipitating cellulose quantitatively, and all methods for estimating cellulose depend on the removal, in one way or another, of the various substances with which the cellulose is usually found in intimate association, the residue being dried and weighed. As these admixed substances are often highly resistant to the most energetic reagents, their complete removal without attack of the cellulose itself is difficult, if possible. Various conventional methods are, however, in use.

Müller's method for the estimation of cellulose in wood, vegetable fibres and substances to be used for the manufacture of paper is as follows: 5 grm. of the finely divided substance is boiled 4 or 5 times with water, using 100 c.c. each time. The residue is dried at 100°, weighed, and exhausted with a mixture of equal volumes of benzene and strong alcohol, to remove fat, wax, resin, etc. The residue is again dried, and boiled several times with water to every 100 c.c. of which 1 c.c. of strong ammonia has been added. This treatment removes colouring matter and pectous substances. The residue is further bruised in a mortar, if necessary, and is then treated in a closed bottle with 250 c.c. of water and 20 c.c. of bromine water containing 4 c.c. of bromine per litre. In the case of the purer bark-fibres, such as flax and hemp, the yellow colour of the liquid disappears only slowly, but with straw and woods decolourisation occurs in a few minutes. When this takes place, more bromine water

is added, the addition being repeated till the yellow colour remains and bromine can be detected in the liquid after 12 hours. The liquid is then filtered, and the residue washed with water and heated to boiling with 1 litre of water containing 5 c.c. of strong ammonia. The liquid and tissue are usually coloured brown by this treatment. The undissolved matter is filtered off, washed, and again treated with bromine water. When the action seems complete, the residue is again heated with ammonical water. The treatment is sufficient with the purer fibres, but the operation must be repeated as often as the residue imparts a brownish tint to the alkaline liquid. The cellulose is thus obtained as a pure white substance. It is washed with water, and then with boiling alcohol, after which treatment it may be dried at 100° and weighed.<sup>1</sup>

**Cross and Bevan's Chlorination Method.**—This method (*J. Chem. Soc., Trans.*, 1889, 55, 199), which gives maximum yields of cellulose, is carried out as follows: 5 grm. of the fibre dried at 100° is boiled for 30 minutes with 1% sodium hydroxide solution, well washed on a gauze filter, squeezed to remove excess of water and placed in a beaker into which a slow stream of washed chlorine gas is passed. The fibre changes in colour from brown to golden-yellow; after 30 to 60 minutes' exposure it is removed, washed and heated to boiling in 2% sodium sulphite solution, 0.1% of sodium hydroxide, calculated on the weight of the solution, being then added and boiling continued for 5 minutes. The cellulose is filtered and washed and is then almost white; it may be finally bleached by immersion in dilute hypochlorite or permanganate solution (0.1%). The amount of cellulose thus obtained is 2 to 5% higher than that yielded by Müller's process and the method far less tedious.

Herig (*Cellulosechem.*, 1921, 2, 34) has devised a convenient apparatus for use with the Cross and Bevan method, and Heuser and Casseus (*Papierfabr.*, 1922, 20, 80) show that this method may be simplified and brought more under control by treating the ground material with a solution of chlorine in carbon tetrachloride.

<sup>1</sup> Briggs (*Analyst*, 1915, 40, 107) points out that, for all practical purposes, "dry" cellulose may be obtained, without appreciable loss of weight due to decomposition, by heating to constant weight in dry air at 105°; cellulose may also be dried completely in 20 hours by heating it at 35° over phosphoric oxide under reduced pressure. When cellulose has been washed with water and subsequently with alcohol, the latter cannot be expelled completely by heat.

Johnsen and Hovey (*J. Soc. Chem. Ind.*, 1918, **37**, 132 *T*) prepare wood for the chlorination process by preliminary digestion at 135° with a mixture of acetic acid and glycerol, the sample being first powdered to pass through an 80-mesh but not through a 100-mesh sieve. According to Dore (*J. Ind. Eng. Chem.*, 1920, **12**, 264), however, all processes involving preliminary hydrolysis in the determination of cellulose by the chlorination method give low results.

Lange's method (*Z. physiol. Chem.*, 1890, **14**, 283) consists in hydrolysing the non-cellulose by means of potassium hydroxide and has been subjected to modification in various particulars. Parker (*J. physical Chem.*, 1913, **17**, 219) finds that the yield of normal cellulose varies with slight changes in the time of boiling, concentration of alkali and temperature.

Other methods involve prolonged digestion with nitric acid and potassium chlorate or with dilute nitric acid at 50° to 80°, and are of subordinate interest.

By virtue of their lignone components, which are unsaturated aldehydes and ketones, lignocelluloses are highly reactive. The magenta colouration they yield with dimethyl-p-phenylenediamine has been adopted as a quantitative measure of the proportion of lignocellulose present in admixture with non-reacting celluloses. The red condensation product formed with phloroglucinol in presence of hydrochloric acid has been investigated by Cross, Bevan and Briggs (*Ber.*, 1907, **40**, 3119), who found that the colour phase involves only about 10% of the total phenol reacting and that constant values are obtainable for the phloroglucinol absorption values of different fibres.

The amount of furfural yielded by fibres when boiled with hydrochloric acid is estimated by Tollens' method, as described under Pentoses.

Methoxyl is estimated by boiling the fibre substance with concentrated hydriodic acid (*Zeisel's method*).

To determine cellulose, lignin and cutin in crude fibre, König (*Zeitsch. Unters. Nahr.-Genussm.*, 1906, **12**, 385-395) digests the fibre in the cold with hydrogen peroxide in presence of ammonium hydroxide, the treatment being continued for a long time with successive additions of hydrogen dioxide until the residue is colourless. This treatment oxidises the lignin and converts it into soluble products. The residue, consisting of cellulose and cutin, is treated with the

cuprammonium solvent to dissolve the cellulose, the cutin remaining unattacked. The liquid is filtered on a Gooch asbestos filter and the cutin residue weighed, the cellulose being precipitated by alcohol and weighed and the weight of crude fibre less the weight of these two taken as lignin.

The results of König's investigations on hay and bran show that lignin contains 55.3 to 59% and cutin 60 to 75.4% of carbon. Cellulose from the same source contains methoxyl groups varying in proportion from 0.4 to 2.82%. Similar methoxyl groups were not found by Cross, Bevan and Beadle in the cellulose from jute. The lignin contains not only methoxyl groups, but also ethoxyl and acetyl residues.

*Estimation of Crude Fibre.*—In valuing foodstuffs a distinction is made between the digestible and indigestible constituents. As the process of animal digestion is in reality an exhaustive series of alternately acid and alkaline hydrolyses, a standard method of estimating the crude fibre has been adopted, consisting in boiling the material first with sulphuric acid and then with sodium hydroxide. For the A. O. A. C. process see page 72.

For the filtration of the acid and alkaline solutions in the estimation of crude fibre, Fanto and Nikolitsch (*Zeitsch. anal. Chem.*, 1915, **54**, 73) recommend the use of a filter thimble capable of holding the whole of the solution. When the liquid has passed out through the thimble, the latter is suspended in water, which traverses the walls of the thimble. This is then raised, and so on. This procedure prevents clogging of the walls of the thimble by the fine particles of the substance.

*Agar-agar.*—This substance, often called Japanese or Bengal isinglass, is prepared from marine algæ and exhibits colloidal properties. It consists of a carbohydrate, sometimes called gelose, which is a polymeride of galactose and is converted into galactose when boiled with dilute mineral acids. Tollens uses the more correct term *d*-galactan for gelose. It has the formula  $(C_6H_{10}O_5)_n$ , and is at first lævorotatory in warm aqueous solution, but becomes dextro-rotatory when subjected to prolonged warming. Agar yields also a small proportion of pentoses when hydrolysed.

Samec and Ssajevič (*Compt. rend.*, 1921, **173**, 1474) regard agar-agar as a sulphuric ester of gelose, and Neuberg and Ohle's results (*Biochem. Zeitsch.*, 1921, **125**, 311) also indicate the existence of

sulphur in organic combination, hydrogen sulphide being evolved by the action of bacteria on agar-agar and sulphuric acid liberated by its hydrolysis.

It occurs in translucent strips of the thickness of a straw or in shorter and thicker yellowish white pieces. It is odourless and tasteless, insoluble in cold water, soluble in hot water. On cooling, the solution gelatinises to a thick jelly which does not melt as readily as that from gelatin. The solution in 500 parts of water still gelatinises when cold. It is employed in the preparation of culture media for bacteria and is used also as a thickening agent in milk and cream and as a substitute for white of egg.

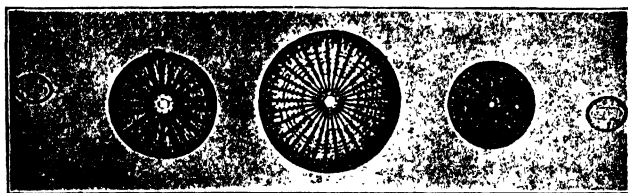


FIG. 87.—*Arachnoidiscus Ehrenbergii*.  $\times 100$ . The smaller oval diatoms are a species of *Cocconeis*. (Löffmann.)

The aqueous solution should give no precipitate with tannic acid solution, proving the absence of gelatin, and no blue colouration with iodine, proving the absence of starch.

Commercial agar usually contains diatoms, a characteristic form being *Arachnoidiscus Ehrenbergii* (see Fig. 87). In order to separate the diatoms, the organic material is destroyed by oxidation with a mixture of nitric and sulphuric acids.

The presence or absence of agar-agar in a foodstuff cannot, however, be presumed from the presence or absence of diatoms, since, on the one hand, some samples of agar-agar contain only few diatoms and, on the other, kieselguhr is sometimes used as a filtering material, especially for fruit juices. A. E. Parkes (*Analyst*, 1921, **46**, 239) gives, for the detection of agar-agar in jam or raw fruit pulp, a method based on treatment with water at  $50^{\circ}$  and on the gelatinising power of a solution of the residue in boiling water in case agar-agar is present.

Agar is mostly exported to China to be used as food and is also much used for thickening cheap ice-cream, especially that sold in American cities under the name "Hokey-Pokey." It is adapted

for such use because its jelly does not liquefy at as low a temperature as that made with gelatin. Agar has also been offered as a substitute for gelatin in ordinary diet because, being practically indigestible and not irritating, it is supposed to give bulk to the contents of the intestine and so promote the peristaltic movements.

## GUMS

Gums are amorphous substances occurring in trees or plants, from which they are derived either by spontaneous exudation or by extraction with solvents. They are perfectly non-volatile, have little or no taste, are uncrystallisable, and eminently colloidal. By virtue of their colloidal properties, gums serve as retarders in the setting of plaster of Paris, and as constituents of mixtures introduced into steam boilers to prevent deposition of hard scale. These characters render their purification very difficult, and hence but little is known of their chemical relationships. For convenience, various pectous bodies are classed with the gums.

The results of O'Sullivan's investigations on gum arabic, Gedda gum and gum tragacanth have shown that the gums are, not carbohydrates of the formula  $(C_6H_{10}O_6)_n$ , as was at one time supposed, but glucosidic derivatives of certain complex organic acids, the latter being united to various hexose, pentose, and biose groups; the composition of the gum from any one plant varies with the season of the year. Further a natural gum is often a mixture of several gum compounds, differing in the number of sugar residues in their molecules.

Various gum substances, *e. g.*, bassorin, arabin and cerasin, have been isolated, but they appear to vary in their characters when obtained from different sources.

Gums are either soluble in, or swell up in contact with, cold water, a character which distinguishes them from starch, cellulose and resins. They differ from the sugars by being incapable of fermentation by yeast, and from the sugars and resins by their insolubility in alcohol. From dextrin the gums soluble in water are distinguished by their laevorotatory power and acid reaction, and by yielding mucic acid on treatment with moderately concentrated nitric acid. Fragments of wood, containing only traces of wood-gum, when boiled with hydrochloric acid and orcinol, show the reaction quite distinctly. From

erythrodextrin and starch the gums differ by giving no colour with iodine solution, and from proteins by yielding no ammonia when heated with soda-lime.

The gums of which gum arabic is the type are dissolved by cold water and are not readily precipitated by acids. Pectin forms a jelly when its aqueous solution is faintly acidified, whilst gum tragacanth merely swells up without undergoing notable solution when treated with cold water.

According to Voley-Boucher (*Bull. des Sciences Pharmacol.*, 1908, **15**, 394), most gums contain a soluble enzyme capable of decomposing amygdalin; soluble gums are more active than insoluble ones. Enzymes are probably associated with the formation of gums *in situ*, but of this little is understood. Gum arabic usually contains an oxydase and gives a colour reaction with tincture of guaiacum, whereas gum tragacanth is devoid of oxydase.

Summaries of the chemistry of the gums are given by H. H. Robinson (*Report Brit. Assoc.*, 1906 (York), 227), and by Stocks (*J. Soc. Chem. Ind.*, 1918, **37**, 52R).

In analysing gums it is necessary to identify the sugars and the gum acids. The principal constants of a gum acid are the ultimate composition, the neutralising power for bases and the optical rotation. As the acids do not crystallise, to prove their individuality it is necessary to precipitate them fractionally and to compare the constants of different fractions. The amount of mucic acid and of furfural yielded should also be determined. The following results of analyses of some tree gums are due to Huerre (*J. Pharm. Chim.*, 1908, [vi], **27**, 561) and Lemeland (*J. Pharm. Chim.*, 1904, [vi], **9**, 584):

Percentage, calculated on dry gum	Almond tree (hard gum)	Almond tree (elastic gum)	Apricot tree	Plum tree
Soluble in water.....	21.06	8.9	91.17	79.16
Insoluble in water.....	78.94	91.1	8.83	20.84
Loss at 100°.....	15.76	25.0	16.14	15.48
Ash.....	2.34	....	3.39	2.52
Galactans as galactose....	23.7	....	23.6	16.36
Pentosans as arabinose....	54.6	....	48.57	76.35
Total sugars.....	85.0	91.0	78.7	94.8
Sugars identified.....	Arabinose and galactose	Arabinose and galactose	Arabinose	Arabinose

For the detection of gelatin in gums and alimentary substances Trillat (*Compt. rend.*, 1898, 127, 724) gives the following method: the substance is dissolved in water, the insoluble matter filtered off, the filtrate concentrated to a syrup, about 1 c.c. of formaldehyde added and the mass evaporated to a pasty consistency. The residue is taken up in boiling water; if gelatin is present an insoluble deposit of horny appearance remains. The deposition is complete in 24 hours, and the weight of the deposit is determined by decanting off the liquid, breaking up the insoluble gelatin, and washing with water by decantation. The precipitate, which contains no sensible amount of formaldehyde, is dried on a water-bath and weighed. In mixtures of gelatin, gum and sugar, the gelatin may be estimated with an accuracy of 0.5 to 1%.

The method is applicable to the estimation of gelatin in commercial jellies, since animal gelatin is easily separated from natural vegetable jellies. Coagulable albuminoids are first removed by boiling. Other proteins besides gelatin may be estimated by this method and the insoluble precipitates are not affected by dilute acid or alkali or by boiling water or other neutral liquid.

**Gum Arabic.** Gum Acacia.—Gum arabic is the dried exudation from the bark of various species of *Acacia*. Strictly speaking, "gum arabic" is the generic name, "gum acacia" being properly limited to the superior qualities employed in medicine. These are largely obtained from the Soudan. The finest gum arabic occurs in commerce in lumps of various sizes, colourless and full of minute cracks. Gum Senegal forms yellowish or reddish lumps, often of the size of a pigeon's egg, and without the minute cracks of the better varieties; it is less readily soluble than true gum arabic, and its solution soon becomes very dark in colour.

Gum arabic consists essentially of calcium arabate or arabic acid (arabin), which may be obtained pure by dialysing a solution of the gum previously acidified with hydrochloric acid. The colloidal liquid thus obtained is lævorotatory, and is not precipitated by pure alcohol, but is thrown down if a trace of any acid or salt is present. After being evaporated to dryness and heated to 100°, arabin does not redissolve, even in hot water, but swells up to a gelatinous mass, which gradually dissolves on treatment with alkalis or alkaline earths in presence of water, yielding a liquid indistinguishable from the aqueous solution of ordinary gum arabic.

Most varieties of gum arabic—including the Levantine, Senaar, East Indian and Senegal—are lævorotatory, but Australian or Wattle gum is often optically inactive, whilst Gedda gum is dextro-rotatory. Chemically, these gums are analogous to the lævorotatory varieties.

The inferior qualities of gum contain a small percentage of a reducing sugar, which may be removed by treatment with alcohol.

The sp. gr. of air-dried gum arabic ranges from 1.35 to 1.49, but when completely dried at 100° the gum loses about 13% of water, and the sp. gr. increases considerably.

Gum arabic has a very faint odour and a mucilaginous insipid taste. It dissolves slowly in about twice its weight of water, forming a thick transparent mucilage of acid reaction. Gum is slightly soluble in dilute spirit, but quite insoluble in liquids containing more than 60% of alcohol, and is precipitated from its aqueous solution on addition of a large proportion of spirit.

The aqueous solution of gum arabic is not precipitated by neutral lead acetate, but with the basic acetate it forms a white jelly. Its solution is precipitated also by potassium or sodium silicate, borax, ammonium oxalate, mercuric chloride or ferric salts.

Suakim or Talca gum, which is quite brittle, is often not wholly soluble in water, but yields with it a pasty mass of rather strong acid reaction, depositing, when diluted with water, transparent globules, said to consist of metagummic acid, which may be rendered soluble by adding a little lime-water.

The proportions of mucic acid obtainable from the different varieties of gum by oxidation with nitric acid have been determined by Kiliani (*Ber.*, 1882, **15**, 34), who found amounts varying from 14.3%, given by a sample of East Indian gum, to 38.3%, from an Australian sample.<sup>1</sup>

By addition of a saturated solution of aluminium sulphate to one of gum arabic, the adhesive properties of the latter are said to be much increased, owing to the formation of aluminium arabate, whilst calcium sulphate is gradually deposited.

<sup>1</sup> The treatment of the gums with nitric acid was conducted in the following manner: 2 grm. of the powdered sample was digested at 60° with 5 c.c. of nitric acid of sp. gr. 1.2 until the whole became a solid mass saturated with the liquid. Another 5 c.c. of nitric acid was then added and the liquid filtered. The residue of mucic acid was washed thoroughly, dried at 100°, and weighed. The filtrate and washings were evaporated together and again treated with nitric acid, when a further quantity of mucic acid was obtained, whilst a third treatment generally yielded only an additional trace.

The presence of gum arabic in a solution prevents the formation of a number of characteristic precipitates (Lefort and Thibault, *Pharm. J.*, 1882, [iii], 13, 301), a fact which is of importance in toxicological researches. Thus, in presence of gum arabic, dilute solutions of mercury, lead, copper, silver, iron, and arsenic salts do not give precipitates with hydrogen sulphide or alkaline sulphides, though the liquids acquire a colour corresponding with the sulphide which would otherwise be precipitated. The precipitation of calcium phosphate and uranyl ferrocyanide is prevented in a similar manner, whilst in presence of gum arabic the alkaloids are not precipitated by sodium phosphomolybdate, potassium mercuric iodide or tannin.

**Analysis of Gum Arabic.**—Gum arabic should contain not more than about 4% of *ash*. It should be soluble almost without residue in cold water. The solution should be free from *starch* and *dextrin*: it should give no reaction with iodine solution, and (unlike *dextrin*) should be rendered turbid by oxalic acid. The better kinds of gum arabic do not reduce Fehling's solution when heated to boiling with it, any red precipitate being due to the presence of a reducing sugar, small quantities of which exist naturally in certain inferior varieties of gum, though any considerable quantity has probably been introduced as an impurity in an admixture of *dextrin*.

According to Z. Roussin (*J. Pharm. Chim.*, 1868, [iv], 7, 251), *gum arabic* and *dextrin* may be distinguished and separated by means of ferric chloride, which precipitates the former only. The solution is concentrated to a syrup, mixed with 10 times its volume of rectified spirit, and the resultant precipitate washed with rectified spirit and dried. 1 grm. of the dry residue is then dissolved in 10 c.c. of water, the solution mixed with 30 c.c. of proof spirit (50% alcohol), 4 drops of ferric chloride solution (containing 26% of the anhydrous chloride) added, followed by a few decigrams of powdered chalk; after brisk stirring the liquid is left at rest for a few minutes and then filtered. The precipitate is washed with proof spirit, and the *dextrin* precipitated from the filtrate by addition of very strong alcohol. After 24 hours the spirituous liquid is decanted, the *dextrin* dissolved in a small quantity of water, the resultant solution evaporated at 100°, and the residue weighed. The precipitate containing the gum must be dissolved in dilute hydrochloric acid, the arabin precipitated by adding absolute or very strong alcohol, the precipitate washed with spirit and dissolved in water, the solution evaporated, and the residue

weighed. The precipitation of gum arabic from a dilute alcoholic liquid by ferric chloride and chalk is so complete that nothing but calcium chloride can be found in the filtrate, whilst the precipitate similarly produced in a solution of dextrin is perfectly free from the latter substance. By the formation of a cloud on adding ferric chloride alone, the presence of gum arabic is sufficiently demonstrated, whilst the clouding of the filtrate from the iron-chalk precipitate on addition of alcohol proves the presence of dextrin.

Another test by which gum arabic may be distinguished from dextrin is given on page 542. A large proportion of dextrin would be indicated by the dextrorotatory action of the solution, but the divergent optical activities of different samples of natural gum arabic and commercial dextrin prevent quantitative application of the test.

For the separation of gum arabic from *sugar*, Andouard dilutes 10 grm. of the syrup with 100 c.c. of spirit of sp. gr. 0.800, adds 20 drops of acetic acid, and stirs vigorously. After 3 hours the liquid is poured on a double filter, where the gum forms a cake which readily drains. This cake is dissolved in a little water, and the precipitation repeated, the precipitate being washed with alcohol, dried at 100° and weighed. It is then exposed to the atmosphere for 24 hours, when it will have taken up its normal amount of moisture.

Inferior gums are employed largely as thickening agents in calico-printing. Good gum neither tarnishes nor alters delicate colours and does not weaken the mordants. The action of gums on delicate colours may be ascertained by printing a solution of the sample mixed with cochineal-pink or fuchsine upon pure wool; the fabric is then steamed and washed, when, if the gum be pure, there will be no trace of yellowness apparent. Too great an acidity of the gum gives it a solvent action on mordants, and hence renders it unsuitable for use.

The relative viscosities of samples of gum are important characteristics in judging of their quality. They may be tested by making solutions of 10 grm. of each sample in a little warm water, diluting the liquids to 100 c.c., and ascertaining the rate at which the solutions flow from a glass tube drawn out to a fine orifice. A recently prepared solution of gum of the best quality should be used as a standard.

**Gum tragacanth** is the gummy exudation from certain species of *Astragalus*. It occurs in flattened, tear-like masses, strings or curved

bands, which are usually marked with ridges or other indications of lamination.

According to Giraud, gum tragacanth usually contains about 60% of a pectinous body which yields pectic acid if boiled with water containing 1% of hydrochloric acid; from 8 to 10% of soluble gum, probably arabin; 5 to 6% of starch and cellulose; 3% of ash; 20% of water, and traces of nitrogenous substances. The ash is chiefly calcium carbonate.

The characteristic pectinous constituent of gum tragacanth is known as tragacanthin, adracanthin, or bassorin, and is stated to have the composition  $C_{12}H_{20}O_{10}$ .

Tragacanth is usually white or yellowish (having sometimes been bleached by chlorine), but the inferior varieties have a brownish colour. It is hard, tough and difficult to powder. Tragacanth is odourless and tasteless and insoluble in alcohol or ether. With 50 parts of water it swells up and forms a thick, jelly-like mucilage, without actually dissolving. When diffused through a much larger quantity of water it forms a ropy liquid which may be passed through a filter, leaving an insoluble residue giving a blue colour with iodine owing to the presence of starch. Mucilage of tragacanth is coloured yellow by sodium hydroxide, and a solution of the gum yields clear mixtures with borax, alkaline silicates, and ferric chloride, but is precipitated by alcohol. It becomes thick on addition of neutral or basic lead acetate, and a precipitate is formed when the mixture is heated.

Before being used for calico-printing, gum tragacanth is swelled by soaking in cold water for 24 hours, and afterwards boiled with water for 6 hours, when a thick homogeneous solution results, which, however, has but little cohesive power. The comparative viscosity of the liquid may be ascertained as in the case of gum arabic (page 561).

To distinguish gum acacia from gum tragacanth advantage is taken of the fact that the latter contains no active oxydase such as is present in gum acacia. A cold aqueous solution (1 in 30) of the gum, is treated with an equal volume of 1% aqueous solution of guaiacol, 1 drop of hydrogen peroxide solution being then added and the mixture shaken. In presence of gum acacia the liquid rapidly acquires a brown tint, whilst with pure gum tragacanth it remains colourless (Paget, *Ann. Chim. anal. appl.*, 1905, 10, 63).

For detecting adulteration of gum tragacanth with gum arabic, Thevenon (*Ann. Falsif.*, 1920, 13, 489) treats a small portion of the sample, mixed with water, with an equal volume of 4% pyramidon (dimethylaminophenyldimethylpyrazolone) solution and then adds 10 drops of hydrogen peroxide (12 vol.): a blue colouration develops in 5 to 30 minutes, according to the quantity of gum arabic present. By this test it is possible to detect 1 part of gum arabic in 20 parts of gum tragacanth.

The gum of *Cochlospermum gossypium* is remarkable for its power of giving off acetic acid slowly when exposed to moist air or on hydrolysis (H. H. Robinson, *J. Chem. Soc., Trans.*, 1906, 89, 1496). It yields a gum acid (gonadic acid), xylose and galactose. This property of emitting acetic acid is also possessed by the gum of *Sterculia urens*.

## PROXIMATE ANALYSIS OF PLANTS

The analysis of plants, as a rule, resolves itself into the qualitative detection and quantitative separation of some single substance or group of substances. So far as the carbohydrates are concerned the subject has been fully dealt with in the preceding pages; and the alkaloids and similar groups of substances will be treated elsewhere. At the present time, biological methods are much used and it is also becoming customary to test plants for enzymes.

The following scheme, practised in the laboratory of Prof. A. B. Prescott, is intended to facilitate the systematic analysis of vegetable substances. Substances proved by its aid to be present should be isolated by methods specially devised for each individual case. Owing to the very rapid changes which take place in plants after being gathered or on being macerated with water, as a result of enzyme action, it is often advisable to destroy the enzymes immediately; this is conveniently done by dropping the freshly cut plant piecemeal into boiling alcohol.

**Moisture** is estimated by methods given on page 66. The loss of weight may include a little volatile oil.

The percentage of total nitrogen is determined by the Kjeldahl method, and is multiplied by 6.33 to obtain the percentage of proteins. It must not be assumed, however, that all the nitrogen present exists as proteins, the contrary being commonly the case. An outline of the method of estimating the nitrogen existing in various

forms is given by Schulze and Barbieri (*Landw. Versuchs-Stat.*, 1881, 26, 213), but if alkaloids are present they must be isolated by separate means.

**Action of Solvents.**—The substance is submitted to a systematic treatment with solvents and reagents in the manner prescribed in the following tables:

J. M. Albahary (*Compt. rend.*, 1908, 146, 336) has given the following scheme for the complete analysis of vegetable substances. A portion is dried at 100° to obtain the quantity of volatile matter, and is then incinerated to give the amount of total ash. A second portion of the sample is extracted with alcohol; the alcoholic extract is distilled at a low temperature, and the distillate collected in a receiver containing a known volume of standard sodium hydroxide solution and surrounded by a freezing mixture. Determination by titration of the excess of sodium hydroxide gives the quantity of volatile acids, and this, added to the weight of the residue remaining in the distillation flask, gives the weight of the alcohol-soluble substances. Subtraction from the weight of the original material of the sum of the substances soluble and insoluble in alcohol gives the actual amount of the fat, colouring matters, cholesterol and lecithin. The portion of the substance insoluble in alcohol is next digested for 2 days in alcohol acidified with hydrochloric acid. The solution is then poured through a filter and the residue is washed with alcohol. The filtrate and washings are evaporated and the residue weighed, extracted with ether to remove organic acids and then dissolved in water. Portions of the solution are used for the estimation of the reducing sugars, mineral acids, nitrogen, asparagine, sulphur and ash. In the portion insoluble in acidified alcohol are estimated the total protein, nuclein, albumin, starch, cellulose, etc.

The following books will be found to give useful special information. For general morphological and microscopic character of plants see Wiesner, *Die Rohstoffe des Pflanzenreiches*; for microscopic identification of technical fibres see Franz von Höhnelt, *Die Mikroskopie der technisch-verwendeten Faserstoffe*.

Treat 5 grm. of the finely-divided substance with benzene, wholly distilling below  $86^{\circ}$ , or, failing this, with chloroform. The treatment should be continued for 6 hours, and be conducted in a Soxhlet's extractor or other suitable apparatus for repercolation.

**Volatilised.**—Volatile oils, camphors (partially), volatile alkaloids. The last may be detected by the alkaline reaction of the aqueous liquid and their loss avoided by adding a drop of hydrochloric acid before evaporation.

**Residue.**—Treat with a moderate quantity of warm water, and when cold filter through fine paper by suction.

**Solution.**—Divide into two equal portions, *a* and *b*:

(*a*) Evaporate to dryness, and weigh total extract. Ignite, and weigh ash.

(*b*) Test portions for alkaloids and glucosides by special reagents; and for organic acids by solutions of barium, calcium, iron, lead and silver salts.

**Residue.**—Remove from the filter and vessels used by benzene or chloroform, agitate solution with warm, very dilute hydrochloric acid, and separate by means of a separating funnel.

**Acid Solution.**—Test for alkaloids and glucosides.

**Benzene Solution.**—Evaporate to dryness, and treat residue several times with spirit of sp. gr. 0.848. Filter through paper.

**Solution** may contain camphors, resins, chlorophyll, certain fixed oils (*e. g.*, castor oil). Camphors are recognisable by the smell; chlorophyll by its absorption spectrum.

**Residue** consists of fixed oils, fats, wax, and, very rarely, resin.

(A) SOLUTION IN BENZENE OR CHLOROFORM.—Evaporate carefully to dryness and weigh the residue. Then treat with water; again evaporate to dryness at 100°, heat to 110°, and weigh again.

(A) <i>Solution</i> may contain alkaloïds, glucosides, free organic acids, chlorophyll, certain resins, fixed oils, fats, and waxes, camphors, volatile oils, but no mineral matter.	(B) <i>Solution</i> may contain mineral matters, tannin, organic acids, alkoloids, glucosides, certain extractive and colouring matters, resins, and sugars.	(C) <i>Solution</i> may contain soluble proteins, gum; and, in the analysis of fruits and fleshy roots, pectin substances, salts of organic acids, dextrinoid substances and colouring matters.	(D) <i>Solution</i> may contain dextrin and maltose from conversion of starch; also proteins and occasionally organic acids, either as salts or free.	<i>Residue</i> .—Wash thoroughly, dry at 110° and weigh. Boil for 2 hours with 500 c.c. of 2% sodium hydroxide solution. Filter through washed linen.	<i>Residue</i> .—Wash thoroughly, dry at 100°, and weigh. Then treat with 500 c.c. of water and 5 c.c. of concentrated sulphuric acid and heat till a drop of the liquid gives no colour with iodine.	<i>Residue</i> .—Dry at 100°, weigh and treat with cold water. Macerate, with frequent agitation, for 8 or 10 hours. Then filter through fine washed linen or paper if possible.
			(E) <i>Solution</i> may contain protein matters, pectinous matters, cutose, humus, and products of decomposition.	<i>Residue</i> .—Wash thoroughly with hot water, alcohol and ether in succession. Dry at 110° and weigh. Treat as directed on page 564.		
					(F) <i>Solution</i> .—Lignin and colouring matter.	<i>Residue</i> .—Weigh as cellulose.

(B) SOLUTION IN ALCOHOL OF SP. GR. 0.848.—Concentrate to a small volume, and remove, dry and weigh any crystals or powder which may separate from cooled liquid. Dilute the clear liquid to 200 c.c. with alcohol of sp. gr. 0.848, and divide into several aliquot parts (20, 20, 20, and 160 c.c.)

20 c.c.—Evaporate to dryness, and weigh total extract. Ignite and weigh ash and gain to estimate ash and total organic extract.	20 c.c.—Evaporate nearly to dryness; add water, filter, and evaporate filtrate to dryness. Residue extract, and on ignition leaves the soluble ash.	<p>(a) Evaporate nearly to dryness, add water, filter and make up filtrate to 100 c.c.</p> <p><b>Residue</b> may contain resins, colouring matters; proteins; especially from seeds; alkaloids and glucosides.</p> <p><b>Solution</b>.—Divide into eight portions of 20 c.c. each.</p> <ol style="list-style-type: none"> <li>1. Precipitate tannin with ammoniacal zinc acetate. The loss of weight on careful ignition of the weighed precipitate dried at 120° represents tannin.</li> <li>2. Add neutral lead acetate. Loss of weight on ignition represents tannic, gallic, and other organic acids, colouring and extractive matters and, rarely, proteins.</li> <li>3 and 4. Precipitate by basic lead acetate, and treat as in 2. After separating lead, treat one-half of filtrate with Fehling's solution to estimate dextrose; invert other portions and determine dextrose; the difference gives dextrose formed from glucosides and sucrose.</li> <li>5 and 6. Treat with basic lead acetate and filter. Decompose both precipitate and filtrate with hydrogen sulphide, testing the former for organic acids, and the latter for alkaloids and glucosides.</li> <li>7 and 8. Use in case of accident to other portions.</li> </ol>	<p>(b) Evaporate carefully to dryness, pulverise and treat residue with several considerable portions of absolute alcohol (sp. gr. 0.7938). Filter.</p> <p><b>Solution</b>.—Evaporate nearly to dryness and add water.</p> <p><b>Residue</b> may contain:</p> <ol style="list-style-type: none"> <li>1. Alkaloids, glucosides (rarely); and extractives soluble in dilute hydrochloric acid.</li> <li>2. Matters insoluble in dilute hydrochloric acid.</li> <li>3. Acid resins and colouring matters.</li> <li>4. Neutral resins, colouring matters, and nitrogenous matters insoluble in dilute ammonia.</li> </ol>	<p><b>Solution</b>.—Add basic lead acetate. Loss of weight on igniting precipitate represents tannin, organic acids and some extractives. Filter, contain alkaloids, glucosides, extractive and colouring matters.</p> <p><b>Residue</b>.—Treat with dilute hydrochloric acid. Dissolve; some alkaloids and glucosides. 2. Insoluble; some resins, and colouring and extractive matters. Dissolve in alcohol; evaporate to dryness, and weigh.</p>
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(C) SOLUTION IN COLD WATER.—Make up liquid to known volume and divide into aliquot portions.

1. *Total solid matter* obtained by evaporating and drying residue at  $110^{\circ}$ ; *ash* by ignition.
  2. Add solution of iodine. Blue indicates *soluble starch*; reddish-brown, *erythro-dextrin*.
  3. Add ammonium oxalate. A white precipitate indicates calcium, probably as calcium arabinate.
  4. Evaporate a known volume, apply Kjeldahl method, and multiply nitrogen found by 6.33 to obtain proteins.
  5. Add dilute hydrochloric acid. A gelatinous precipitate consists of *pectin* or *pectic acid*; if the liquid be filtered and treated with 4 times its volume of alcohol, a further precipitate may consist of *arabin* or *dextrin*.
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(D) SOLUTION IN DILUTE ACID.—Boil with excess of barium carbonate, exactly neutralise last traces of acid by cautious addition of solution of barium hydroxide, filter, concentrate, and bring volume to exactly 50 c.c. Then ascertain sp. gr., and divide excess above 1000 by 8. The figure thus obtained is the weight of starch in the 5 grm. of substance taken. If the sp. gr. indicates but a small proportion of starch, treat half the solution with 1 c.c. of concentrated sulphuric acid, and heat the liquid to  $100^{\circ}$  for 3 or 4 hours; then neutralise, and estimate glucose by Fehling's solution. The amount found, multiplied by 0.9, gives *starch*. Test portion of original neutralised solution by adding tannin. A white or buff precipitate indicates *proteins*.

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(E) SOLUTION IN DILUTE ALKALI.—Add slight excess of hydrochloric acid. A precipitate may contain *pectic acid* and other substances, such as colouring matters. Further precipitation usually occurs on addition of alcohol.

## CEREALS

Owing to the defective methods employed, many of the older analyses of wheat and other grains are of doubtful value.

A. H. Church gives the following results of analyses by himself in illustration of the composition of representative specimens of the cereal grains and products therefrom:

	White English wheat	Fine wheat flour	Wheat bran	Scotch oat- meal <sup>1</sup>	Pearl barley <sup>2</sup>	Rye flour	Cleaned rice	Maize	Millet	Dari
Water.....	14.5	13.0	14.0	5.0	14.6	13.0	14.6	14.5	13.0	12.2
Proteins and other nitro- genous bodies.....	11.0	10.5	15.0	16.1	6.2	10.5	7.5	9.0	15.3	8.2
Starch with traces of dex- trin, etc.....	60.0	74.3	44.0	63.0	76.0	71.0	76.0	64.5	61.6	70.6
Fat.....	1.2	0.8	4.0	10.1	1.3	1.6	0.5	5.0	5.0	4.2
Cellulose and lignose.....	2.6	0.7	17.0	3.7	0.8	2.3	0.9	5.0	3.5	3.1
Mineral matter.....	1.7	0.7	6.0	2.1	1.1	1.6	0.5	2.0	1.6	1.7
	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

<sup>1</sup> One hundred pounds of oats yield about 60 of oatmeal and 26 of husks, the remainder being water and loss.

<sup>2</sup> The product called pearl barley constitutes only about one-third of the whole seed.

For convenience of comparison, the following analyses of other vegetable products are given. They are selected from among a large number published in Church's work on *Food*:

	Buck- wheat	Peas	Haricot beans	Lentils	Earth- nut (shelled peanut)
Water.....	13.4	14.3	14.0	14.5	7.5
Proteins.....	15.2	22.4	23.0	24.0	24.5
Starch.....	63.6	51.3	52.3	40.0	11.7
Fat.....	3.4	2.5	2.3	2.6	50.0
Cellulose and lignose.....	2.1	6.5	5.5	6.9	4.5
Mineral matter.....	2.3	3.0	2.9	3.0	1.8
	100.0	100.0	100.0	100.0	100.0

	Pota- toes	White turnips	Carrots	Beet- root, red	Yam
Water.....	75.0	92.8	89.0	82.0	78.6
Proteins.....	2.3	0.5	0.5	0.4	2.2
Sugar.....	.....	.....	4.5	10.0	} 16.3
Starch.....	15.4	.....	.....	.....	
Dextrin, gum and pectose.....	2.0	4.0	0.5	3.4	
Fat.....	0.3	0.1	0.2	0.1	0.5
Cellulose and lignose.....	1.0	1.8	4.3	3.0	0.9
Mineral matter.....	1.0	0.8	1.0	0.9	1.5

For further results reference may be made to König's *Chemie der menschlichen Nahr.-und Genuss-mittel*, to Leach's *Food Inspection and Analysis*, and to various publications of the U. S. Dept. of Agriculture, Bureau of Chemistry. From the last source the following figures are taken:

## COMPOSITION OF CEREAL GRAINS

	Moist- ure	Pro- teins (6.25 N)	Ether ex- tract	Crude fibre	Ash	Carbohy- drates other than crude fibre
Typical unhulled barley.....	10.85	11.0	2.25	3.85	2.5	69.55
Typical American maize.....	10.75	10.0	4.25	1.75	1.5	71.75
Typical wheat.....	10.6	12.25	1.75	2.4	1.75	71.25
Sweet corn, 19 samples (Richardson).....	8.44	11.48	8.57	2.82	1.97	66.72
Typical American buckwheat.....	12.0	10.75	2.0	10.75	1.75	62.75
Typical unhulled oats.....	10.0	12.0	4.5	12.0	3.4	58.0
Typical rye.....	10.5	12.25	1.5	2.1	1.9	71.75
Typical rice, unhulled.....	10.5	7.5	1.0	9.0	4.0	67.4
Typical rice, hulled but unpolished.....	12.0	8.0	2.0	1.0	1.0	76.0
Typical rice, polished.....	12.4	7.5	0.4	0.4	0.5	78.8

## COMPOSITION OF FLOURS

	Moisture		Ash		Proteins (6.25 N)		Fibre		Ether extract		N-free extract	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
Wheat.....	15.0	9.0	0.8	0.3	15.0	8.0	1.0	0.1	2.0	0.5	90.0	82.0
Rye.....	14.0	12.0	1.5	0.5	11.0	6.0	0.6	0.4	1.0	0.9	92.0	88.0
Barley.....	15.0	10.0	2.0	1.0	12.0	8.5	0.6	0.3	2.0	0.5	92.0	87.0
Buckwheat.....	18.0	12.5	1.5	0.8	9.5	5.0	0.6	0.3	2.0	0.8	93.0	84.0
Rice.....	15.0	10.0	0.6	0.3	10.0	7.0	0.4	0.1	0.6	0.3	90.0	85.0
Oat (meal).....	10.0	6.0	2.4	2.0	18.0	14.0	1.4	0.7	9.5	6.5	76.0	72.0
Maize (meal).....	18.0	8.0	4.5	1.0	11.5	8.0	3.5	0.7	6.0	2.5	80.0	63.0
Graham (wholemeal).....	15.0	11.0	2.2	1.8	15.0	10.0	2.4	2.0	2.2	1.9	72.0	70.0

**Proteins of Cereals.**—Although cereals are composed mainly of starch, it is often the protein constituents which impart to them their characteristic properties and which therefore are investigated in an analysis. Indeed, in very many of the published analyses, the starch is determined by difference. Although different proteins occur in different cereals, many of the proteins contain about 15.8% of nitrogen and it has become usual to deduce the proportion of proteins present by multiplying the percentage of nitrogen by 6.33  $\left[\frac{100}{15.8}\right]$ . This procedure ignores the fact that the whole of the nitrogen of plants does not exist in the form of proteins and may be very misleading if the analysis is used to judge of the suitability of a cereal for bread-making or of its food value. For wheat proteins the factor 5.7 is considered more accurate.

This subject will be dealt with fully in Volume VIII.

During recent years much attention has been paid to those indispensable accessory food factors known as vitamins, which are considered in a subsequent volume of the present work. A concise account of this subject is given in a paper on "Vitamins and Food Supply" by Harden (*J. Soc. Chem. Ind.*, 1921, 40, 79R), who discusses, *inter alia*, the effect of milling on the vitamin-content of grain.

## WHEAT

Two varieties of wheat—*Triticum vulgare*—are cultivated, distinguished as spring and winter wheat. Individual species and samples differ rather widely in composition. König's table for the mean percentage composition of wheat (from 250 analyses) is as follows:

Water,	13.56
Proteins,	12.42
Fat,	1.70
Starch,	64.07
Sugar,	1.44
Gums and dextrin,	2.38
Fibre,	2.62
Ash,	1.79

## FLOUR

Flour consists of the finely ground interior of the wheat grain, and is produced from wheat by a complicated series of processes of "conditioning" (this, known also as "dampening" or "tempering," consists in wetting the wheat some time before grinding), grinding between rollers, and separation of the bran, germ and fibre by means of sieves or air-draughts. Wheat yields about 70% of pure flour, but in some cases the aim is to effect only imperfect removal of the bran and the germ, the grades known as wholemeal or standard flours being thus obtained.

Flour should be an impalpable powder with only a faint yellow tinge, but as regards colour it must be borne in mind, firstly, that some strong flours, *e. g.*, Russian, are darker than others, and secondly, that bleaching of flour is practised very generally. Storage for 1 or 2 months after milling whitens and improves the baking qualities of flour but more prolonged storage causes deterioration and increase in acidity.

No mites or other parasites visible to the naked eye or under a lens should be present, and when pressed smooth by means of a polished surface, flour should exhibit no traces of bran; a suspected sample is sometimes sifted through a fine silk sieve and the residue examined.

The odour should be pleasant; that of a flour showing signs of unsoundness may be accentuated by boiling a little of the material with water containing a small proportion of sodium hydroxide. An agreeable taste and freedom from acidity are also desirable.

Flour is sometimes subjected to elaborate adulteration and conditioning, in order to increase the weight by admixture with foreign starches, etc., to increase the "strength" by addition of "improvers," and to enhance the whiteness and uniformity by bleaching.

The baking value of flour depends on its strength, *i. e.*, its ability to produce a large-volumed well-risen loaf, and also on its water-absorbing capacity. Many investigations have been made on the strength of flour but the causes of this quality are still imperfectly understood. In general, flour containing a large proportion of gluten furnishes a better loaf than flour with only a small proportion, yet certain flours are known which have little gluten and are, nevertheless, possessed of good loaf-producing properties. One of the most important contributions dealing with this question is that

made by T. B. Wood (*J. Agric. Sci.*, 1907, **2**, 139, 267), who considers that strength is the outcome of two factors, one determining the size, and the other the shape of the loaf. These factors depend respectively on (1) the amount of carbon dioxide evolved in the dough, and (2) the consistency of the gluten, this being conditioned, not by its composition, but by the salt-content and acidity of the medium with which it is in equilibrium. When suspended in certain salt-acid solutions gluten becomes tough and stable, whereas in others it disintegrates and loses all coherence. Thus, the acids and salts present in an aqueous extract of flour undoubtedly exert a marked effect on the consistency of the gluten, so that their variations may furnish a clue to the abnormal behaviour of certain flours.

On the other hand, the results obtained by Gortner and Doherty (*J. Agric. Res.*, 1918, **13**, 389) show that the rates of swelling or hydration of gluten in different solutions depend largely on the nature of the gluten itself, and not merely on the concentrations of the ions in the solution. Gluten from a "weak" flour has a much lower rate of hydration than that from a "strong" flour and changes from a *gel* to a *sol* at a much lower degree of hydration. It may be that in weak glutes the particles approach more closely to the boundary line between the colloidal and the crystalloidal states; in any case a strong gluten has the emulsoid properties of a perfect colloidal *gel* to a far greater extent than a weak gluten. Moreover, Rousseaux and Sirot (*Ann. Falsif.*, 1917, **10**, 556; *Compt. rend.*, 1918, **166**, 190) find that the ratio of the soluble to the total nitrogen in a flour may be regarded as an index of its baking value, the most favourable proportion being about 16 to 17%; if the ratio is higher, owing to the preponderance of soluble nitrogenous matter, the baking quality is poor, whereas if it is lower, owing to the presence of more insoluble and therefore probably more complex nitrogen compounds, the flour is worked only with great difficulty.

In this connection, attention may be directed to the series of papers by Ostwald and Lüers (*Koll. Zeitsch.*, 1919, **25**, 26, 82, 116, 177, 230; 1920, **26**, 66) on the colloidal chemistry of flour and bread. Viscosity measurements of suspensions of wheaten flour (up to 20%) in water show that such suspensions are extremely sensitive to traces of acids, which greatly increase the viscosity; sodium chloride appears to reduce it. A flour of poor baking quality was found

to give viscosities considerably below those given by normal flours. Further, experiments on the viscosity of gliadin solutions appear to indicate that changes in the gluten and in the baking quality of flour are dependent largely on changes occurring in the gliadin constituent of the gluten; the viscosity of gliadin solutions is greatly affected by changes of temperature and the temperature at which the viscosity reaches a maximum is also that at which the best dough and bread are produced. The addition of a trace of acid, which increases the viscosity of the gliadin solution, likewise enhances the quality of the bread.

According to Martin (*J. Soc. Chem. Ind.*, 1920, 39, 246, 348T), the gas-retaining capacity of gluten, which increases with the strength of the flour, is associated with the gliadin constituent of the gluten. Bailey and Weigley (*J. Ind. Eng. Chem.*, 1922, 14, 147) utilise the loss of carbon dioxide per unit increase in volume of dough under definite conditions as a measure of the gas-holding capacity of the dough and consequently of the strength of the flour.

All flours contain a catalase, which is present to the greatest extent in the offals, and Marion (*Compt. rend.*, 1920, 171, 804) finds that an idea of the degree of extraction of a flour is given by the volume of oxygen generated in a given time from a certain amount of hydrogen peroxide by a definite quantity of the flour. Marotta and Kaminka (*Giorn. Chim. Ind. Appl.*, 1922, 4, 249) assert that the results of this test are of value only when the original grain is available and comparison with flours of different percentage extractions thus possible.

Many chemicals, including some of a highly dangerous nature, have been used at different times as "improvers"; they are usually added by atomising aqueous solutions with the flour. The most common "improvers" are alum, copper sulphate, persulphates, and phosphoric acid and its acid salts. The addition of phosphates has been defended as being advantageous in view of their nutritive value, but this contention is applicable only to phosphorus in organic combination, in which form it is usually present when occurring naturally in foods.

The bleaching of roller-mill flour originated in England about 1906 and has since become general. Several bleaching agents have been employed, such as ozone, chlorine, oxides of chlorine, bromine, and oxides of nitrogen, the last being most commonly used. The

amount of  $N_2O_4$  in the air with which the flour is treated averages 40 to 100 parts per 1,000,000 and the amount used to bleach 1 kilo of flour varies from 3 to 50 c.c. The  $N_2O_4$  absorbed reacts quantitatively with test reagents for nitrates.

**Microscopic Examination of Flour.**—By this means such substances as foreign starches, fibre, germ and ergot may be detected. A convenient method of examining flour or other solid food product for starches consists in placing a minute quantity of the material on a slide and mixing thoroughly with a very small drop of glycerol by means of a glass rod drawn out fine and fused at the end to make a small bead. A cover-glass is then pressed firmly down on to the preparation, the amount of glycerol being insufficient to exude from beneath the cover-glass.

**Colour.**—Small wedge-shaped heaps or parallelepipeds with a smooth surface are prepared from the flour and from a standard flour for comparison, and are arranged side by side on a sheet of glass and the colours compared when dry and after careful immersion of the plate in water, when, as a rule, the colours become much more marked.

**Doughing Test.**—A known weight of the sample is made into a dough with one-half to two-thirds its weight of water and the colour and “feel,” that is, firmness, elasticity and compactness, of the doughs are compared.

The proportion of water necessary to convert a flour into dough which does not stick to the fingers is sometimes determined.

**Baking Test.**—The relative values of different flours for making bread are sometimes ascertained by carrying out small laboratory baking tests under constant conditions, the shapes and volumes of the various loaves obtained being compared.

**Gluten Test.**—To obtain results comparable at different dates much care must be paid to detail in carrying out this test. About 30 grm. of flour is made to a stiff dough with 12 to 15 c.c. of water and allowed to stand for 1 hour. The mass is then carefully kneaded in a stream of running water until all the starch has been removed. This kneading is conveniently carried out between the fingers, the flour being held over a sheet of fine muslin which allows the starch to pass but retains any particles of gluten which may fall on it. Other operators advise kneading the flour wrapped in a piece of linen. The ball of fresh gluten thus obtained should be tough and elastic,

capable of being pulled out in threads and but little coloured. Flour from English wheats gives a very soft and sticky gluten showing very little elasticity, whereas that from Canadian<sup>1</sup> spring wheats is very tough and elastic. After washing, the gluten is left for an hour under water, the excess of moisture being then removed between the hands as far as possible and the wet gluten weighed. It is then dried for 40 hours at 98° C. or for a shorter period at a higher temperature and the weight of dry gluten determined.

With low-grade flour or with mixed flour it is sometimes impossible to obtain a coherent cake of gluten; disintegration during the washing process may, according to Bouyer (*Bull. Soc. Pharm. Bordeaux*, 1919, No. 2), be prevented by immersing the cake for a few seconds in ether. The washing and kneading are then continued, with further treatment with ether if necessary.

This crude gluten consists in reality of true gluten together with small percentages of non-gluten proteins, mineral matter, fat, starch, fibre, etc.

### ANALYSIS OF FLOUR

**1. Moisture** is determined by heating the sample at 100° until no further loss in weight occurs. The keeping qualities depend, *inter alia*, on the percentage of moisture in the flour, this being normally about 13, but varying from about 10 to 15.

**2. Fat** is determined by extraction of the dry flour with ether, and varies in the same direction as the ash. A high value for the ether extract of a patent flour (above 1.5%) indicates incomplete removal of germ particles.

**3. Gluten** may be estimated approximately by washing as described above or by a nitrogen determination on the original flour. The percentage of nitrogen found is multiplied by the factor 5.7 (in the case of wheat this is preferable to 6.33). The proportion of dry gluten in flour usually lies between 8% and 12%, being dependent on the quality of the wheat and on the season.

**4. Ash** is best determined in a muffle or the flour may be mixed with ammonium nitrate and burnt. If above 1%, either mineral adulteration or low-grade flour is indicated.

**5. Starch** may be estimated by one of the methods already described; see page 527. It varies inversely with the ash, nitrogen,

<sup>1</sup> Or Northwestern. United States. Amer. Eds.

fat, cellulose and soluble carbohydrates, the limits being about 60 to 72%.

6. **Gliadin** may be determined by extracting with 70% (by volume) alcohol for 2 hours and determining nitrogen in the filtrate. This is multiplied by the same factor as the gluten nitrogen.

7. **Cold water extract**, which consists of sugars, soluble proteins, potassium phosphate, etc., is obtained by digesting the flour with a large volume of water, filtering and evaporating an aliquot portion in a weighed dish. The residue is ignited to give the soluble ash. The result depends largely on the temperature and time of extraction, as under these conditions the diastatic properties of a flour cause a rapid increase in the amount of soluble sugar.

8. **Acidity** is best determined by direct titration with decinormal alkali, using phenolphthalein as indicator. Normal wheat has an acidity 0.16 to 0.25%, calculated as lactic acid. 20 grm. of flour is shaken with 200 c.c. of water for 2 hours, filtered and 50 c.c. of the filtrate titrated. The test is most useful for detecting unsound wheat and flour.

9. **Diastatic Power**.—A mixture of 0.4 grm. of the flour and 200 c.c. of a 2% solution of soluble starch is maintained for an hour at 15.5°. Action is stopped by a drop of ammonia and portions of 2 c.c. are heated in test-tubes in boiling water for 5 minutes with different quantities of Fehling's solution to determine the amount which is just reduced by the sugar formed. The diastatic power is 100 when 2 c.c. reduce 4 c.c. of Fehling's solution. It ranges in commercial flours from 25 to 60. Ling's method (*see* Malt) may also be used.

**Gluten** consists of two proteins: gliadin, remarkable for its solubility in dilute alcohol, and glutenin, which is soluble in very dilute alkali. Gliadin in the hydrated condition is a soft, sticky substance which can readily be drawn into threads; when dehydrated it forms a white friable mass. It may be prepared by extraction of flour or gluten with 70% (by volume) alcohol and precipitation from this solution by sodium chloride. It is readily soluble in pure water. Gliadin forms a sticky mixture with water, and the salts naturally present in the flour prevent its solution. The glutenin imparts solidity to the gluten, evidently forming a nucleus to which the gliadin adheres.

The ratio of gliadin to total gluten varies in different flours and authorities are not agreed as to the correlation of this ratio with

strength. A flour with a high gliadin ratio is considered the best: Fleurent suggests 75%, Snyder 60%, but these values are not accepted by British authorities.

**Mineral Constituents of Wheat and Flour.**—Whole wheat contains a much higher percentage of ash than flour does. The ash of wheat ranges from 1.4 to 1.9%, and that of good flour seldom exceeds 0.7% or 0.8% in the case of seconds flour. It may safely be assumed that any flour which is free from a notable proportion of bran and which yields a higher ash than 1% is adulterated.

Blyth quotes the following table as the mean composition of the ash of entire wheat:

	WINTER WHEAT	SUMMER WHEAT
Potassium oxide	31.16	29.99
Sodium oxide	2.25	1.93
Calcium oxide	3.34	2.93
Magnesium oxide	11.97	12.09
Ferric oxide	1.31	40.51
Phosphoric oxide	46.98	48.63
Sulphur trioxide	0.37	1.52
Silica	2.11	1.64
Chlorine	0.22	0.48

According to Snyder, the ash of wheat flour bears a relation to the grade—the lower the grade the higher the percentage of ash. First patents have less than 0.4%, second patents less than 0.5% and a straight grade flour should not have more than 0.55% of ash. Mixing or misbranding of flours may be determined more certainly in this way than by any other means. The figures quoted refer to hard Canadian spring wheat.

The United States standard of purity is not more than 13.5% of moisture, 1% of ash and 0.5% of crude fibre and not less than 1.25% of nitrogen.

**Detection of Bleaching Agents in Flour.**—In many of the processes (those of Alsop and Andrews) employed for bleaching flour use is made of small quantities of nitrogen oxides in air, with or without ozone, as bleaching agent. Others involve the use of chlorine or bromine. In all cases of bleaching, the agents probably form additive products with one or more of the constituents of the flour. One of the most delicate tests for nitrites is the Griess-Ilosvay test (see page 303),

which may be applied as follows: 20 to 30 grm. of the flour is extracted with an equal number of c.c. of benzene and the solution filtered into a porcelain dish. If the filtrate is colourless or nearly so, bleaching may be suspected. The solution is evaporated to dryness and the colour of the oily residue observed. An orange-red often indicates nitric oxide, whilst chlorine or bromine give a faint yellow or nearly white residue. An ignited bead of copper oxide is moistened in the oily residue and held in the bunsen flame, a bright green tinge confirming chlorine or bromine. 20 c.c. of water is added to the exhausted flour residue and 1 c.c. of the Griess-Ilosvay reagent added, a very characteristic pink resulting if nitrous bleaching agents have been employed. The occasional use of nitrosyl chloride as a bleaching agent makes both tests desirable.

Buchwald and Neumann (*Z. ges. Getreidewesen*, 1909, 135) make use of a reagent obtained by dissolving 0.5 grm. of sulphanilic acid and 0.1 grm. of  $\alpha$ -naphthylamine in two separate portions of 150 c.c. of 30% acetic acid (in vessels previously rinsed with acetic acid) and then mixing the two solutions, any red colouration appearing either at once or after long standing being removed by addition of zinc dust. From 3 to 5 drops of this reagent are placed on a little of the flour spread out and pressed flat, bleached flour developing a pink or red colour after the lapse of a minute at latest, whereas untreated flour remains uncoloured.

Another excellent reagent for the detection of nitrites is that devised by Lombard (*Bull. Soc. Chim.*, 1913, [IV], 13, 304): 1 grm. of sulphanilic acid is dissolved in 100 c.c. of hot saturated ammonium chloride solution, 1.5 grm. of phenol and then 100 c.c. of approximately 2N-HCl being added. 5 grm. of the flour is macerated in a porcelain mortar with 100 c.c. of water, no bunsens being alight near. The mixture is filtered and 50 c.c. of the filtrate mixed in a 100 c.c. cylinder with 1 c.c. of the reagent, 1 c.c. of ammonia solution (sp. gr. 0.880) being added after 15 minutes and the liquid stirred. In the presence of nitrites, an orange-yellow colour will be produced, varying in intensity directly with the proportion of nitrites present. For quantitative purposes, a comparison is made with the colour given under similar conditions with a standard nitrite solution.

Unbleached flour frequently absorbs nitrite from the air, especially in industrial towns, but the amount then taken up is well within

0.7/10<sup>6</sup>. During storage a decrease in nitrites may occur, with a corresponding increase in nitrates. For further information reference may be made to *Reports to the Local Government Board on Public Health and Medical Subjects, New Series, No. 49, Food Report, No. 12, 1911*: "On the Bleaching of Flour and the Addition of So-called Improvers to Flour," by Dr. J. M. Hamill; and "On the Chemical Changes Produced in Flour by Bleaching," by Dr. G. W. Monier-Williams (Wyman & Sons, London).

**Detection of Improvers and Adulterants in Flour.**—Flour may be adulterated with mineral matters to increase its weight, with alum or copper sulphate to improve its appearance, or with cheaper flours or starches. In exceptional cases it may contain extraneous seeds or have been damaged by mould and contain ergot.

The amount of *ash* affords a convenient and accurate means of detecting mineral adulterants with the exception of alum, which is usually employed in too small a quantity sensibly to affect the percentage obtained. The mineral adulterants may be separated from a flour by shaking it with chloroform in a separating funnel and leaving it till the flour has risen to the surface. Any mineral adulterant sinks in the chloroform and may be removed and examined. It is further purified by a second treatment with chloroform; the residue is obtained on a watch-glass, the chloroform removed by evaporation, and the solid weighed.

Alum or other crystalline matter is detected by microscopic examination; the residue is dissolved in a little cold water and filtered and the insoluble matter ignited and weighed. It should not exceed 0.1%, if the flour is free from insoluble mineral adulterant. The solution is evaporated to dryness and any crystals of alum observed; they may be tested for aluminium, sulphates, potassium and ammonium or the alum may be recognised by its astringent taste and by its reaction with logwood.

**Logwood Test for Alum.**—1 grm. of freshly cut fine logwood chips is digested for 10 hours in 30 c.c. of alcohol; 10 c.c. of this extract is mixed with 150 c.c. of water and 10 c.c. of a saturated solution of ammonium carbonate; 50 grm. of flour is made into a thin paste with water, a few drops of the *fresh*<sup>1</sup> alkaline logwood solution added and the

<sup>1</sup> In employing the logwood test for alum, it is very important that the tincture of logwood should be freshly prepared, and that the test should be made immediately after mixing the logwood tincture with the solution of ammonium carbonate. In attention to these essential points has resulted in failure to obtain the blue colouration with specimens undoubtedly containing alum. Further, the subsequent drying should never be neglected.

mixture put aside for some hours. Alum produces a lavender-blue lake, whilst pure flour gives a pinkish colour which fades to a dirty brown. The test is sensitive to  $\frac{1}{10,000}$  part of alum. The blue colour should remain when the sample is heated for an hour or two in the water-oven.

Wynter Blyth uses small strips of gelatin on which to concentrate the alum. A strip is soaked for 12 hours in the cold extract of the suspected flour and then taken out and steeped in the ammoniacal logwood, when, if alum is present, it acquires a very marked blue colour. The strips may be washed and dissolved in hot water and the absorption spectrum of the solution observed.

The logwood test is given only by aluminium salts in solution, no blue colouration being produced by the aluminium normally present in flour as a silicate. The alumina normally present in a pure flour bears a fairly constant relationship to the silica present, the latter being approximately equal to the alumina calculated as alum. Thus it is customary to calculate alumina as alum, to diminish the latter by the amount of the silica and to regard the remainder as adulteration, subject to confirmation by the logwood test.

Alum increases the whiteness and improves the apparent quality of inferior flour. Its presence in flour or bread is always to be regarded as a sign of adulteration (see under "Bread").

**Copper sulphate** may be detected, even when present in but very minute proportion, by soaking the flour in a solution of potassium ferrocyanide acidified with acetic acid, when a purplish or reddish-brown colouration is produced if copper is present. The amount of copper may be estimated by moistening 100 grm. of the flour with sulphuric acid, igniting and estimating the metal in the ash.

Very minute proportions of copper have been stated to exist normally in wheat-ash.

**Persulphates.**—20 grm. of the flour are extracted with 100 c.c. of water, the liquid filtered, and 10 c.c. of a 2% solution of benzdine in alcohol added to the filtrate; a blue colour is formed with dilute persulphate solution, or a yellow with concentrated solution.

**Phosphates.**—Phosphate "improvers" are detected by the presence of abnormally high percentages of phosphate in the ash and

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With proper care, the test is exceedingly delicate, 0.02% of alum causing a distinct shade of blue, whilst with three or four times this proportion the reaction is wholly beyond question.

On the other hand, blue colouration of bread and flour by an ammoniacal solution of logwood does not infallibly prove the presence of a soluble aluminium compound, as several other mineral additions produce a somewhat similar reaction.

in the soluble extract; when they are suspected, analysis of the ash is essential.

**Plaster of Paris** is readily separated from flour by treatment with chloroform.

**Ergot in Flour.**—The use of mouldy wheat in manufacturing flour may often be detected by moistening the sample and keeping it in a tightly closed vessel for some hours at about 30°, when any mouldy taint is readily detectable.

To test for ergot or other fungus, Vogel advises microscopic examination of the flour after staining with aniline violet. Starch granules that have been injured by the fungus acquire an intense violet tint, whereas sound granules remain relatively colourless. Grüber heats a little of the moistened flour on a microscopic slide to the b. p. of water and examines with a power of 120 diameters when cold. Ergot may be identified by its high refracting power, furrows and colour—deep violet on the edge, greenish-yellow inside.

Chemical tests for ergot are as follows: (1) 20 grm. of flour are exhausted with boiling alcohol in a Soxhlet or other suitable apparatus until the last extract is colourless, and 1 c.c. of cold dilute sulphuric acid then added. In the presence of ergot the solution will be red and when examined by the spectroscope in dilute solution will show two absorption bands: one in the green near *E*, and a broader and stronger band in the blue between *F* and *G*. On diluting the alcohol with a large volume of water the colour may be extracted from separate portions by ether, amyl alcohol, chloroform and benzene.

2. 10 grm. of flour are digested for half an hour with 20 c.c. of ether and 10 drops of dilute sulphuric acid (1:5). The liquid is filtered and the residue washed with ether until 15 c.c. of filtrate are obtained. This is shaken with sodium hydrogen carbonate, which takes on a deep violet colour if ergot is present, whereas the chlorophyll remains in the ether.

**Wood-meal (Sawdust).**—For the detection of wood-meal in flour Le Roy (*Ann. Chim. anal.*, 1899, 4, 212) suggests the following test: 1 grm. of phloroglucinol is dissolved in 15 c.c. of 90 to 95% alcohol and 10 c.c. of syrupy phosphoric acid; 1 to 2 c.c. of this reagent are rubbed with a little of the sample in a porcelain dish, wood-meal assuming at first a rose and gradually a carmine tint.

Another method consists in spreading out the flour on a flat bottomed porcelain dish, compressing it and then gently heating it with

0.1% alcoholic phloroglucinol solution acidified with 50% sulphuric acid; any woody particles are immediately coloured carmine, cortical residues of the wheat becoming coloured after some time. Paganini (*Chem. Centr.*, 1905, **1**, 695-696) moistens the flour spread in a thin layer first with 0.2% aqueous paraphenylenediamine solution and then with acetic acid. The particles of wood-meal are at once coloured orange-yellow.

To detect rice flour, Gastine (*Compt. rend.*, 1906, **142**, 1207) advises staining with aniline blue or green, which shows up the hilum of the minute rice-starch granule as a reddish point.

Ivory-nut meal (Fig. 80) may be detected by the following test, due to Bertarelli (*Accad. reale di Medic. Torino*, 1906): the flour is treated with 3% sodium carbonate solution and left for 30 minutes, after which the liquid is decanted off and the residue washed with water and examined under the microscope. The ivory-nut meal is readily recognised by its characteristic structure, the cells of the endosperm of this seed exhibiting an enormous thickening of the walls, so that the aperture of the cell is distinctly reduced. These thickenings are interrupted here and there by channels connecting the cells.

In Gerum's test (*Zeitsch. Unters. Nahr.-Genussm.*, 1914, **5**, 392), 5 grm. of the flour are shaken in a separating funnel with chloroform and left at rest for 6 hours. Any ivory-nut meal present is then deposited on the bottom of the funnel and may be detected microscopically.

**Foreign Flours.**—The addition of other flours to wheaten flour is somewhat uncommon, but may occur. The detection of such addition is a matter of considerable difficulty and requires patient examination under the microscope. Various investigations, particularly those of Bigelow and Sweetser, Kraemer and Vogel, have established certain data on which tests may be based.

Vogel advises extraction with 70% alcohol containing 5% of hydrochloric acid. Pure wheat or rye flour gives a colourless extract, with barley or oats a pale yellow extract and with pea flour an orange-yellow extract is obtained, whilst mildewed wheat gives a purple-red and ergotised wheat a blood-red colouration.

Amberger (*Zeitsch. Unters. Nahr.-Genussm.*, 1921, **42**, 181) finds that, at 58-59°, wheat, rye, barley and oat starches are readily saccharified by diastase, whilst maize, potato and bean starches remain unaffected and may be detected microscopically among the

fragments of tissue in a flour; the small rice starch granules are also revealed in this way.

Much may be ascertained from the gluten, which is dark and viscous when rye flour is present, dark, non-viscous and dirty reddish-brown with barley, dark yellow with oats, yellowish and non-elastic with maize, and varies from a greyish-red to green in the case of leguminous flours, such as bean or pea.

Leguminous starches give more ash than wheat flours; this ash is deliquescent and rich in chlorides, and turns turmeric paper brown. To detect legumin, the gluten is washed from a sample of the flour in the usual manner and the filtrate made alkaline with ammonia. It is allowed to stand overnight, the clear liquid being then decanted and the legumin precipitated by a dilute mineral acid. It may be collected, dried and weighed. According to Lemenant des Chenais, 0.9 gm. of legumin may be taken as indicating the presence of 5% of leguminous flour.

A method of detecting potato flour in wheat is based on the resistance to destruction of the outer membrane shown by wheat flours. The sample is rubbed in a mortar with water to a stiff paste, which is then diluted and filtered. The filtrate, tested with a drop of dilute iodine solution, gives a deep blue with potato starch and a yellow or orange with pure wheat flour.

Another method, due to Donné, consists in making a microscopic preparation of the flour in the ordinary way and passing dilute potassium hydroxide under the cover-glass; under this treatment potato starch granules gradually swell until they reach 4 to 5 times their original volume, whilst the wheat starch remains almost unaffected.

According to Wallis (*Pharm. J.*, 1922, **109**, 82) wheat starch may be definitely recognised in a flour by the presence of a number of granules measuring  $40\mu$  or more; for the quantitative analysis of mixed flours microscopically the pure starches employed for comparison must be prepared by a standardised process, which must be used also for the separation of starch from the mixed flour.

Kraemer states that 5% of maize in wheat flour may be detected by mixing 1 gm. of the sample with 15 c.c. of glycerol and heating to boiling for a few minutes. Maize is indicated by the well-known odour of pop-corn.

If a small quantity of the flour is treated with 10 c.c. of 1.8% potassium hydroxide solution for 2 minutes in a test-tube and then nearly neutralised with hydrochloric acid, wheat starch is gelatinised, whilst maize remains intact.

According to Abel (*Zeitsch. Unters. Nahr.-Genussm.*, 1920, 39, 44), rye flour, which contains no gluten, prevents the recovery of the calculated amount of gluten when added to wheat flour; thus, mixtures of wheat and rye flours containing 0, 10, 20, 30, 40, 50 and 60% of the latter gave respectively 9.0, 8.1, 7.2, 6.0, 4.5, 2.8, and 0.6% of dry gluten. Similarly, mixtures of wheat and barley flours containing 0, 10, to 80% of the latter were found to give 9.0, 8.9, 9.2, 9.1, 9.0, 8.3, 6.2, 3.5, and 0.1% of dry gluten.

Vogt (*Zeitsch. Unters. Nahr.-Genussm.*, 1921, 42, 145) finds that reliable indications may be obtained from: (1) Differential staining by means of a dilute solution of Congo-red in Indian ink, followed by microscopic examination. (2) the "true alkalinity" of the ash prepared in presence of a known volume of approximately N/10-sodium carbonate solution. This true alkalinity, which represents the total alkalinity of the ash towards methyl orange, less that of the phosphates present—determined by titration with alkali hydroxide solution—is expressed as the number of c.c. of N/1-alkali per 100 grms. of dry matter, has a value between -5 and -15 for wheaten flours, and increases with the degree of extraction of the wheat; for barley, oat and maize flours the average value is -20, for wheat offals -31, for potato starch -5, and for potato flocks and rolled potato flour +20 to +25.

## BREAD

Bread is the flour of wheat made into a paste by kneading with water and permeated with carbon dioxide, produced as a rule by fermentation with yeast, but also by other methods. On baking, the gluten swells and retards the escape of the gas, which expands little cells and gives to bread its familiar light, spongy appearance. The outside of the loaf becomes as hot as 210°, and is to some extent caramelised; the inside crumb is seldom raised much above 100°. The amount of water in a loaf varies from 30 to 40% on the average, as is shown by the following analyses of wheaten bread collected by König and quoted by Wynter Blyth.

	Minimum	Maximum	Mean for fine bread	Mean for coarse bread
Water.....	26.39	47.90	38.51	41.02
Nitrogenous substances.....	4.81	8.69	6.82	6.23
Fat.....	0.10	1.00	0.77	0.22
Sugar.....	0.82	4.47	2.37	2.13
Carbohydrates.....	38.93	62.98	49.97	48.69
Fibre.....	0.33	0.90	0.38	0.62
Ash.....	0.84	1.40	1.18	1.09

The moisture depends, among other conditions, on the quality and quantity of the gluten and the size and shape of the loaf.

The ash of a wheaten flour loaf seldom exceeds 1.5%; beyond 2% would indicate mineral adulteration. A small quantity of common salt is usually added to bread during manufacture.

In the Third Report on Colloid Chemistry and its General and Industrial Applications (*H. M. Stationery Office*, 1920), Whymper considers colloid problems in bread-making; the properties of the most important materials used in bread-making—starch, gluten, mineral salts and enzymes—are discussed and attention is drawn to the very great influence which additions of milk, fat, etc., exert on the physical nature and keeping qualities of bread.

Bread is not often adulterated. To test for alum, bread is moistened with water and then with alkaline logwood solution if alum is present the bread will become lavender-blue in an hour or two. The crust and crumb should be analysed separately, as an alumed flour has been known to be used for dusting and facing the sponge before baking it. To search for alum in crust, the latter must be burnt to ash.

Blyth has found that a certain proportion of the alum may always be washed out of the bread as alum. 100 grm. of bread is soaked in water for about 24 hours, the liquid strained through muslin and concentrated in a platinum dish. A strip of gelatin is steeped in a portion overnight and the logwood test applied, a blue colouration being obtained if alum be present to the extent of 0.03%. Allen suggested dissolution of the starch by malt extract, removal of soluble carbohydrates by yeast, acidification with nitric acid, filtration, evaporation, ignition of the residue and precipitation as phosphate in the usual way.

Alum is estimated by the Dupré-Wanklyn method as follows: 100 grm. of bread is ashed in a platinum dish, the ash boiled with 3 c.c. of strong hydrochloric acid and 30 c.c. of water, the liquid filtered and the precipitate (chiefly silica and unburnt carbon) washed, dried, burnt and weighed. 5 c.c. of ammonia is added and the calcium, magnesium, iron and aluminium phosphates precipitated. The liquid is *strongly acidified* with acetic acid, boiled and filtered, and the insoluble phosphates remaining are washed and dried and weighed. The precipitate is redissolved and the iron estimated colourimetrically with ammonium sulphide, calculated as phosphate and this subtracted from the total to give the weight of the aluminium phosphate.

An alternative method consists in burning the ash, boiling with hydrochloric acid and filtering as above. The filtered solution is again boiled and poured hot into a very strong solution of sodium hydroxide, the mixture being again boiled and filtered whilst hot. A little disodium hydrogen phosphate is added to the filtrate, which is then slightly acidified with hydrochloric acid and finally made just alkaline by ammonia. The precipitate of aluminium hydroxide is filtered, washed, ignited and weighed.

Flour normally contains a small proportion of aluminium in the form of silicate. It is customary, therefore, to determine the silica in the bread, to subtract it from the amount of alum calculated from the aluminium phosphate found and multiply the remainder by 3.87 or 3.71 to give approximately the potassium or ammonium alum, respectively. (For further information on this question see Blyth's "Foods.")

The use of porcelain vessels is to be avoided throughout the process and care taken that the alkaline liquids are not heated in glass and that the sodium hydroxide used is scrupulously free from alumina.

The presence of plaster of Paris in bread is recognised by the high total ash and the high proportion of calcium contained in it. The sulphates of the ash do not afford a means of determining accurately the amount of gypsum present, as proteins furnish a notable quantity of sulphates when the cereals are ignited. On the other hand, only traces of sulphates exist ready formed in the cereals, and hence the estimation of them in the unignited bread affords a means of measuring the gypsum present. This method, though theoretically perfect, is complicated by the difficulty of obtaining a solution of

the sulphates fit for precipitation with barium chloride. The best way is to soak 12.20 grm. of the bread for some days in 1200 c.c. of cold distilled water till mould commences to form on the surface of the liquid. The solution is strained through coarse muslin, and the filtrate treated with 20 c.c. of phenol distilled over a small quantity of lime. The whole is then raised to the b. p. and filtered through paper. 1000 c.c. of the filtrate are then slightly acidified with hydrochloric acid, and precipitated in the cold by barium chloride. 1 part of barium sulphate represents 0.583 part of plaster of Paris.

The presence of potato flour in bread may be detected by taking advantage of the fact that the potato starch takes up certain coal-tar colours, such as thionin, neutral-red or methylene-blue, more rapidly than wheat or rye starch (Schütz and Wein, *Chem. Zeit.*, 1915, 39, 143).

Legendre (*Ann. Falsif.*, 1917, 10, 293) gives the following method for deciding if a bread has been made from a particular flour: 2 grm. of the flour and 3 grm. of the bread crumb are placed in separate test-tubes, 10 c.c. of water and 10 c.c. of phosphoric acid (sp. gr. 1.514) are added to each tube and the mixtures are heated at 120° in an autoclave for an hour. When cool, the sediments are collected on a silk sieve (120 mesh), washed with water, alcohol and ether, and then dried and weighed. These weights, when calculated on equal weights of dry matter in the flour and the bread, should be identical. When a rapid indication is required, it is sufficient to centrifuge the two sediments with water and to note the volumes; these should be equal if 2 grm. of the flour and 3 grm. of the bread are used.

The determination of the hydrogen ion equivalence is of growing importance in bread making (see AMS. ED.

### MACARONI, VERMICELLI, SPAGHETTI, NOODLES, ETC.

The quality of these materials is determined principally by that of the flour, usually hard (durum) wheat flour, used in their preparation.

Rancidity or mouldiness is indicated by the taste and smell, the presence of mould or parasites by naked eye or microscopic examination, by which means also flour other than wheat flour may be detected (see Figs. 77 and 78). Tests should be made for heavy metals (copper, lead, zinc, etc.) and for alum, which is sometimes added, together with sodium bicarbonate, to prevent acidification.

These products are often coloured with organic colouring matters or saffron, sometimes with the object of imitating the colouration produced by addition of eggs. In the United States and in Italy and other European countries the use of picric acid, Victoria yellow, Martius yellow and metanil yellow for this purpose is forbidden. The presence of a coal-tar colour may be detected by Possetto's method (*Giorn. Farm. Chim.*, 1914, 390): to 250 c.c. of boiling water contained in a porcelain dish are added first 20 c.c. of 95% alcohol and 2 c.c. of 10% ammonia solution and immediately afterwards 30 grm. of the material. After about 5 minutes' boiling—when it is considered that the liquid is sufficiently coloured—cold water is added and the solid allowed to settle. The liquid is decanted into another dish and, after slight acidification with 10% hydrochloric acid, a small skein of defatted wool (0.5 grm.) boiled in it for 10 minutes. If the wool remains yellow after repeated washing with water, the presence of a coal-tar colour is indicated. Possetto also describes a method for ascertaining if such colour is one of the prohibited ones (see also Piutti and Bentivoglio, *Gazzetta*, 1906, 36, [ii], 385).

The presence of egg is often indicated by the taste and colour—artificial colouring being assumed absent. The following tests may also be made: (1) the finely ground material is heated in a reflux apparatus with light petroleum and ammonium carbonate, the material being afterwards filtered and the filtrate shaken with methyl alcohol, which is then evaporated on a water-bath; the odour of the residue indicates either the presence or the absence of egg. (2) The percentage of fat is increased by the presence of egg, and (3) the iodine number of the fat is diminished, that of the oil of wheat being above 100 and that of egg fat about 70 to 80. A quantitative indication may be obtained in either of the following two ways: (1) by determination of the phosphoric acid of the lecithin; this amounts to 0.02 to 0.03% of the dry matter of the macaroni when egg is absent, and increases by approximately 0.02 to 0.025% for each egg added per 100 grm. of flour used in the preparation. Noetzel (*Zeitsch. Unters. Nahr.-Genussm.*, 1921, 42, 299) finds that the alcohol-soluble phosphoric acid in flour cannot be estimated by a single extraction of the flour with absolute alcohol for 15 hours, and that the extracted residue should be mixed with water to a moderately thick paste and this dried in a thin layer at 100°, finely ground

and extracted again with the alcohol for 12 hours. (2) By determination of the cholesterol, the quantity of the latter present in an egg being 0.17 to 0.25 grm. (see Juckenack and Pasternak, *Zeitsch. Unters. Nahr.-Genussm.*, 1900, 3, 13, and 1904, 8, 94).

### BISCUITS AND MILK FLOUR

The principal components of biscuits are flour and sugar, with sometimes fat (butter, etc.) and eggs. Milk flour should contain flour, sugar and the constituents of milk, although the latter are sometimes quite absent.

The character of the flour used in making the biscuit or milk flour may be ascertained by microscopic examination, and the percentages of moisture, ash, fat, sugar (saccharose, invert-sugar, glucose and lactose), dextrin, starch and nitrogenous matters may be determined. Artificial sweetening substances and colouring matters may be tested for by the usual methods.

For the estimation of the sugar in baked articles, such as biscuits, buns, cakes, etc., a method was selected at a conference between the Government Chemist and representatives of the Society of Public Analysts and of Biscuit Manufacturers (see *J. Soc. Chem. Ind.*, 1917, 36, 856).

### OTHER CEREALS

(For composition see page 592)

**Maize (*Zea Mays*).**—Maize or Indian corn is extensively cultivated in North America, Argentine, South Africa, various Asiatic countries, the Danubian district, etc., and is used largely as a food for human beings and animals. In Great Britain it is consumed as corn-flour in food for infants and invalids and is also utilised in large quantities in breweries and in patent still distilleries. It is relatively rich in oil, which is sometimes extracted and utilised as salad oil, as a lubricant and in soap making.

**Oats (*Species of Avena*).**—Oats are grown in northern regions throughout the world and also in Bengal and the United Provinces. They contain about 6% of fat and a high proportion of mineral matter and are largely used as horse feed. When freed from the husk and converted into groats or oatmeal, they are largely used as breakfast foods in some countries and are sometimes adulterated with

other cereals, notably barley; such admixture is detectable only microscopically. Oats are sometimes malted and used in distilling.

**Barley (*Species of Hordeum*).**—Barley is grown chiefly for making malt but is used also for cattle food. Barley meal and “pearl barley,” which is the grain deprived of its outer coating and rounded by attrition, are used as foods, and barley meal is sometimes used as an adulterant in other foods.

**Rye (*Secale Cereale*).**—Rye is largely cultivated in Central and in Northern Europe and to some extent in Great Britain. Among Northern European nations rye bread forms a staple article of diet, but in Britain rye is used principally in yeast distilleries, sometimes as malted rye. It is comparatively poor in fat, which consists largely of triolein. The grain is particularly susceptible to attack by ergot.

**Rice (*Oryza Sativa*).**—This is cultivated throughout the East and also in Central America and the Carolinas, Texas and Louisiana, and, in spite of its comparative lack of proteins (7 to 10%), constitutes the main food of a large fraction of the human race. In the husk it is known as “paddy,” but it is marketed in Europe mostly as pearly grain free from husk.

To improve its appearance, rice is commonly polished with talc. Excess of the latter is regarded as prejudicial to health, and it has been suggested that the permissible limit for mineral matter in rice should be 0.5%.

*Estimation of Talc.*—5 grm. of the rice are treated in a flask with 5 c.c. of 3% hydrogen peroxide solution, 2 c.c. of ammonia solution (1 vol. of sp. gr. 0.880 and 3 vols. of water) and 3 c.c. of water. To loosen the talc the whole is heated in a steam-bath for some seconds, shaken intermittently for 10 minutes and the liquid with loose mineral matter decanted into a small beaker; the residue is washed 10 times by shaking with 10 c.c. portions of water. To the total liquid 10 c.c. of concentrated nitric acid are added and the whole boiled gently for 10 minutes. The whole is filtered through an ashless filter-paper and the residue ignited at a dull red heat and weighed.

**Bananas.**—These combine the sweet qualities of a fruit with the nourishing properties of a vegetable; they are rich in sugar and starch and contain a fair quantity of proteins. Banana flour is made by drying the ripe fruit and has the following percentage composition: moisture, 9 to 12; fat, 0.32 to 0.44; protein, 3.0 to 4.8; fibre, 0.7 to

0.8; starch, 77 to 83; glucose, 0.9 to 1.8; ash, 1.8 to 2.3; phosphoric anhydride, 0.13 to 0.17 (Eaton, *Agric. Bull. Fed. Malay States*, 1918, 6, 430). Flours prepared from cassava, yams, sweet potatoes and sago are mainly used in the tropics as substitutes for wheat flour.<sup>1</sup> Flours from Morocco beans, mixed American and Plate maize, buckwheat and Algerian millet—which have been used as substitutes for wheat flour in bread-making—have been analysed by Balland (*Ann. Falsif.*, 1918, II, 388).

A number of wheat and oat products are now prepared, particularly in the United States, as breakfast foods. These are either uncooked, or partially cooked by steaming and drying, or malted and cooked. The following analyses of some of these were quoted by Harcourt (*J. Soc. Chem. Ind.*, 1907, 26, 240):

PERCENTAGE COMPOSITION OF SOME BREAKFAST FOODS

	No. of samples analysed	Water	Crude protein	Crude fat	Carbo.* hydrates	Crude fibre	Ash	Energy per grm. (calories)
Granulated oats.....	12	7.75	12.29	6.65	71.71	(1.59)	1.60	4.283
Rolled oats.....	19	8.55	11.83	6.61	71.35	(1.25)	1.66	4.238
Wheat farina.....	8	10.63	9.70	1.05	78.23	(0.62)	0.57	3.876
Wheat germ.....	1	8.39	10.97	2.79	76.77	(1.15)	1.08	4.034
Rolled wheat.....	2	10.41	8.77	1.90	77.22	(2.05)	1.70	3.860
Flaked barley.....	4	10.59	9.71	1.43	76.81	(2.07)	1.47	3.854
Cornmeal.....	2	9.76	6.99	1.26	81.49	(0.52)	0.50	3.870
Orange meat.....	3	8.66	9.70	1.31	78.43	(1.95)	1.90	3.909
Porce.....	3	9.06	10.14	1.51	76.88	(1.85)	2.41	3.886
Norka.....	3	7.38	14.33	5.55	69.91	(1.84)	2.83	4.229
Malta Vita.....	3	8.23	9.88	1.39	78.27		2.23	3.915
Grape Nuts.....	3	7.08	11.49	0.94	78.78		1.71	3.995
Canada Flakes.....	2	8.97	10.84	1.18	76.22		2.79	3.874
Shredded Wheat.....	2	9.41	11.53	0.85	76.51		1.70	3.916
Rice Flakes.....	1	12.29	7.24	0.68	80.04	(0.55)	0.35	3.716

\* Including fibre.

Tibbles, in *Foods, Their Origin, Composition and Manufacture* (London 1912), pp. 440, 467, gives the results of analysis of a considerable number of breakfast, infant and invalid foods.

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<sup>1</sup> Peanut flour was used in the United States to some extent admixed with wheat flour during the war. Amer. Eds.

# PAPER AND WOOD PULP TESTING

BY E. SUTERMEISTER

The methods of testing described in this chapter have been confined to those applicable to the finished paper or to the wood pulps entering into its manufacture. No attempt has been made to describe the methods of paper making, nor has it been thought best to include tests of the inorganic raw materials, such as lime, sulphur, alum, soda, etc., which are necessary for its preparation. The intermediate products and wastes of the manufacturing operations, black liquor, black ash, black ash waste, lime mud, etc., have also been omitted, and for a discussion of these subjects reference must be made to the text books of inorganic analysis and of paper making.

## PAPER

The complete examination of a sample of paper involves the determination of the fibrous constituents and also of a large number of physical and chemical factors. In most cases, however, a comparatively few tests are sufficient to prove the value of a paper for any specific purpose, but those tests must be selected which have a direct bearing upon the use for which the paper is intended. It must also be understood that without a good knowledge of the properties and applications of paper, as well as a considerable experience in carrying out the tests, it is hardly possible to draw sound conclusions from laboratory investigations. For convenience the tests to be applied may be grouped under three headings: (1) microscopical, (2) physical and (3) chemical.

**Microscopical Tests.**—By means of the microscope it is possible to determine the kinds of fibre used in a sample of paper, their relative proportions and something about the treatment they were given in the beater. The accuracy of such an analysis depends on the experience of the observer and on the use of standard samples for comparison. It is far more difficult to estimate the proportions

when three or four kinds of fibre are present than when only two were used, but under average conditions an accuracy greater than 5% is not to be expected. The opinion of one well trained observer is probably more accurate than the average judgment of a number who have had less experience.

The type of microscope to use is largely one of personal choice, but it should be of some standard make so that additional equipment may be added when desired. It should include a substage condenser and iris diaphragm and it is well to add both a stage and eyepiece micrometer. Opinions differ as to the magnification which it is best to employ in examining the fibrous constituents of paper, some advocate 45 diameters, whilst others prefer 120 or even 160 diameters. If the equipment of the microscope includes  $\frac{2}{3}$ - and  $\frac{1}{6}$ -in. objectives and 1- and 2-in. eyepieces the range of magnification will be ample for practically all paper work.

**Estimation of Fibres.**—The first step in estimating the fibres used in a paper is the preparation of the sample, and in order that this may be representative several small pieces should be taken from different parts of the sheet, or if a number of sheets are available, a little piece may be taken from each. In order to remove sizing and binding materials the combined sample should be heated to the b. p. with a little 0.5% sodium hydroxide solution. The solution is then poured off and the samples washed several times in water. They are then rolled into a ball, kneaded between the thumb and finger and finally reduced to a pulp by shaking vigorously with a little water in a test-tube. A small sample is removed from the tube, placed on a microscope slide, stained with appropriate reagents and after covering with a cover-glass is ready for examination under the microscope.

In carrying out these operations a number of precautions should be observed if good results are to be obtained. The more important of these points will be discussed briefly.

In washing the sample after boiling with sodium hydroxide it is often difficult to remove all the alkali; a wash with dilute acid followed by water will overcome this difficulty. The same result may be attained by adding a drop of dilute acid to the test-tube in which the sample is reduced to a pulp.

There are two quite distinct methods for removing a representative sample from the test-tube. The most usual method is to shake

the sample vigorously, insert a microscope needle and take out a small bunch of the fibres. With long fibred stock such as bonds, ledgers, etc., this method gives good results, but if the mixture contains both long and short fibres an unduly high proportion of the former is likely to be obtained. A modification of this method will make it applicable to all types of stock. If the mixture in the test-tube is made with so little water that settling cannot take place it will be found, after shaking the tube and then standing it upright, that small clots of fibre adhere to the walls of the tube above the main mass. One of these clots of the desired size may be removed with a needle and used for the examination.

The second method is one described by Spence and Krauss. (Spence and Krauss, *Paper*, 20, May 23, 1917, 12). They prepare a quite dilute pulp in a large test-tube ( $\frac{3}{4} \times 8$  in.), and for removing the sample use a glass tube slightly less than a quarter of an inch in diameter. One end of this tube is fitted with a rubber bulb and the walls at the other end are rounded to present a smooth surface. When the sample is ready to be removed, the test-tube is thoroughly shaken, the dropper inserted 2 in. below the surface, two bubbles of air expelled and the mixture drawn into the tube to a height of about half an inch. This sample is transferred to slides, making 4 drops when the tube is completely emptied.

In whatever manner the sample is taken the next step is to remove the water previous to staining. This may be done by drying in an air-bath, being careful not to dry so hard that fibres are not readily separated when the stain is added, or a piece of filter paper or blotting paper of firm texture may be pressed down directly onto the sample on the slide. This absorbs the water and leaves the damp fibres adhering to the slide in a good condition for staining. The blotting paper should be free from lint and it is well to remove any possible loose fibres by blowing across its surface before use. Another method is to place the sample taken by the needle directly upon a piece of suitable blotting or filter paper; when the water has been absorbed the sample may be transferred to a slide.

The cover which is placed over the stained sample may be one of the very thin cover-glasses made for such work, but this is only necessary for samples which are to be examined with high power objectives. For all ordinary work a thin microscope slide forms a satisfactory cover and is considerably easier to handle than the cover-

glass. It also has the advantage that it assists in spreading the fibres if it is allowed to fall from a distance of about half an inch onto the drop of liquid in which the fibres have been well loosened up.

Among the stains used for differentiating the various fibres that of Herzberg is very generally employed. This consists of two solutions:

- (A) 20 grm. zinc chloride  
10 c.c. distilled water
- (B) 2.1 grm. potassium iodide  
0.1 grm. iodine  
5 c.c. distilled water

The solutions should be prepared separately, mixed and allowed to stand until all sediment has settled; the clear liquid is then ready for use. It should be kept in the dark or else in brown-glass bottles. This stain gives different colours with the different fibres as follows: Yellow or lemon-yellow: groundwood, jute, flax tow, uncooked

• manila hemp and practically all highly lignified tissues.

Blue or navy blue: thoroughly cooked and bleached soda and sulphite pulps, bleached straw and esparto.

Wine red: cotton and linen fibres, thoroughly cooked and bleached manila hemp and some Japanese fibres.

In using this stain its quality should first be proved by trying it on known samples of fibre, or better, a mixture of about equal parts of rag stock, bleached sulphite and bleached soda. When the stain is satisfactory the soda fibre will be coloured dark blue, the sulphite a lighter blue and the rag fibres wine red. If the blue is not clear but is more of a violet it indicates too much iodine and more water or zinc chloride should be added. A stain which gives the best colour with groundwood is usually unsatisfactory for rag fibres or well bleached wood pulps. If papers of all grades are to be examined it is well to keep several stains on hand. One should be so adjusted as to give a bright lemon-yellow on groundwood and a slightly greenish-blue on unbleached sulphite, whilst a second should be prepared to give satisfactory colours on rag fibres and bleached wood celluloses.

Another stain, used for the same work as the Herzberg stain, is made up as follows:

(A) 1.3 grm. iodine and 1.8 grm. potassium iodide in 100 c.c. of water.

(B) A clear, practically saturated solution of calcium chloride.

To use this stain place a drop of solution A on the moist fibres on the slide; after a minute or two remove the solution by pressing a bit of blotting paper directly onto the sample and then at once put on a drop of solution B. Tease the fibres apart with needles to get them well distributed, and then drop on a thin microscope slide as a cover. If any excess of solution B squeezes out between the slides remove it by means of moist blotting paper. The colours produced by this stain are:

Brownish red to red: linen, hemp, cotton, ramie.

Dark blue: bleached soda pulps from broad-leaved woods.

Reddish or bluish violet: bleached sulphite and the thoroughly cooked portions of unbleached sulphite.

Greenish yellow: manila, jute and the more lignified fibres in unbleached sulphite.

Yellow: groundwood.

This stain should be tried out on known samples and its strength adjusted until satisfactory colours are obtained. The solutions are not much affected by light but they should be protected from dust and evaporation.

In estimating the proportions of the fibres in paper the sample is considered as being made up entirely of fibres and no account is made of the filler, size, alum, etc. The sum of the various percentages of fibres will therefore amount to 100. The estimation should be based on the examination of from two to four slides from the same test-tube, according to the accuracy desired and the importance of the analysis. It is quite essential that a set of standard samples containing known mixtures be available for comparison with unknown samples. These are especially valuable to the beginner and should be used occasionally to refresh the judgment of the more experienced worker.

The percentage of the various fibres in a sample of paper may be determined either by the *count* or the *estimation* method. In the first the number of fibres of each kind is found by actual counting, and the percentages are then calculated. The *estimation* method depends

upon training the eye by examining samples of known composition. Accuracy in this method requires experience and frequent reference to known standard samples. It is to be recommended in preference to the count method on account of its greater accuracy when making groundwood determinations and its equal accuracy under other conditions, and also because of the possibility of comparison with standard mixtures. It is more rapid than the count method and is easier to teach to a beginner. For satisfactory work in fibre estimations by any method it is essential that the analyst should have a thorough knowledge of the size, shape, and characteristic markings of different paper-making fibres as well as of any cells, ducts, etc., which usually accompany them.

Spence and Krauss (*Paper*, 20, May 23, 1917, page 11) have proposed a third method which makes it possible to determine the percentage of each kind of fibre in a mixture of such fibres as spruce, beech, poplar, birch, maple, etc. Four slides are made up and each is examined under the microscope and the lengths of the fibres measured in terms of the diameter of the field. A magnification of 160 diameters will be found satisfactory for this work and a mechanical stage is quite necessary in order that the entire sample may be systematically examined. After measurement of all four slides the figures are added together to get the total length of each kind of fibre present. The total length multiplied by a factor for each kind of wood gives results which are directly comparable and which may be converted easily into the per cent. by weight of each kind of fibre. The factors for various fibres are: poplar pulp 0.454, birch pulp 0.652, beech pulp 0.525, maple pulp 0.365, hemlock pulp 0.870, rag pulp 1.0. A determination of the number of ducts of each kind will show the number of counts to which each weight factor is to be applied. The proportion of fibres and ducts in several kinds of wood is as follows:

Wood	Per cent. fibre	Per cent. ducts
Maple.....	97.7	2.3
Beech.....	95.1	4.9
Birch.....	97.6	2.4
Poplar.....	94.1	5.9

This method is accurate and is recommended as a means of settling disputes; it is too slow to be applied to any considerable number of daily routine analyses.

*Unbleached Sulphite Determination.*—This method, which may be used to distinguish bleached and unbleached sulphites in paper, is described by C. G. Bright (*Paper*, 20, Aug. 29, 1917, 11) in part as follows:

The principle of the method is first to stain the fibres with Cross and Bevan's ferric ferricyanide solution, which colours the unbleached sulphite green, on account of the lignin contained in it, and leaves the bleached sulphite colourless. This alone gives a good distinction, but by subsequently staining with a red substantive dyestuff, the green of the unbleached is changed to a very pure blue, the bleached being coloured red, thus giving a most striking contrast.

The problem is to adjust the treatment with the two solutions to bring out the sharpest contrast. If the treatment with red is too severe some of the unbleached fibres are likely to be coloured purplish, or in extreme cases take on a dull, dirty red colour. On the other hand, if the treatment with ferric ferricyanide is continued for too long a time or at too high a temperature, the reagent has a tendency to decompose and form a deposit on the slide as well as on the bleached sulphite, so that the latter turns a dull purplish colour when subsequently stained with red.

The results depend on three factors, namely: (1) the concentration of the solution; (2) the temperature at which each is applied, and (3) the length of time each is allowed to act.

The solutions are prepared according to the following procedure:

#### FERRIC FERRICYANIDE

Sol. A. N/10  $\text{FeCl}_3$ —2.7 grm.  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  per 100 c.c.

Sol. B. N/10  $\text{K}_3\text{Fe}(\text{CN})_6$ —3.29 grm. per 100 c.c.

After diluting to the mark with distilled water, filter through dry filters into clean, glass-stoppered bottles—protect from dust. Equal volumes are mixed fresh whenever the reagent is used.

#### SUBSTANTIVE RED

Benzopurpurin 4B extra (Bayer Co.)	0.4 grm.
Oxamine brilliant red BX (Badische Co.)	0.1 grm.
Distilled water	100 c.c.

Have the water hot and stir in the dyes slowly.

The stain is placed in a tall, narrow cylindrical beaker, which is set into a water-bath. The slides are suspended in the beaker

by a clamp which holds them at their upper ends, the clamps resting across the top of the beaker. The bath is heated by a small bunsen burner with a pilot flame. The burner can be turned on full to heat the bath up to the required temperature ( $35^{\circ}$  and  $45^{\circ}$ ); then, by turning off the burner and regulating the pilot flame, the bath can be kept at the required temperature without much difficulty. A thermometer is suspended in the beaker of stain beside the slides. The beaker should be as small as possible so as not to use up too much stain at one time.

Staining with ferric ferricyanide is done as follows:

Mix equal volumes of two fresh solutions and heat to  $35^{\circ}$  regulating the water-bath so that it will remain within  $1^{\circ}$  of the temperature named for 15 minutes. The dry slide is then dipped in water to moisten it uniformly, so that air bubbles will not be formed when it is immersed in the stain. If air bubbles are formed, the fibres under the bubbles will not be stained. If dipping in water still leaves bubbles, they can be removed by blowing across the slide from the edge. The slide is then suspended in the stain and left there for 15 minutes at  $35^{\circ}$ . It is then removed and washed by dipping in and out of a beaker of distilled water six times and repeating the process in a fresh beaker of water. The slide can then be placed wet into the red solution, but it is perhaps best to dry it out so that the fibres will be stuck on tightly again, in case they have been loosened to any extent by the treatment.

Staining with the substantive red solution is done as follows:

A fresh solution is heated to  $45^{\circ}$ , and the slide, after moistening and excluding bubbles as before, is suspended in the solution for 5 minutes at  $45^{\circ}$  and immediately washed in two beakers of distilled water.

The slide is then dried and a cover-glass placed on with a drop of balsam.

To get the clearest, brightest results, distilled water must be used throughout, and the staining solutions must be fresh. The two solutions for ferric ferricyanide will keep well if placed in separate bottles. Equal volumes are mixed together immediately before using. The red solution should be made fresh each time for the best results, as it gets thick and stringy on standing, especially when it is being heated up continually.

This method of staining will, in general, give a distinction between pure cellulose fibres and those which contain lignin. Rags, bleached sulphite, soda pulp or any thoroughly bleached material are stained red, while unbleached sulphite, groundwood, jute, or any lignified materials are stained blue.

**Sulphate Fibre.**—A method for distinguishing between unbleached sulphate and sulphite fibres is described by Lofton and Merritt (*Technical Assn. Papers*, Dec., 1920, Series iii, as follows:

The stain which was found to be most satisfactory in differentiating between unbleached sulphite and sulphate pulps, or fibres, was a mixture of 1 part of a 2% aqueous solution of malachite green and 2 parts of a 1% aqueous solution of basic fuchsin, or magenta. The solutions were made up according to the following formulas, kept in tightly stoppered separate bottles, and mixed only when wanted for use:

(A) Malachite green.....	2 gramm.
Distilled water .....	100 c.c.
(B) Basic fuchsin.....	1 gramm.
Distilled water.....	100 c.c.

Since there is considerable variation in the quality of dyes from various sources, it is not to be expected that any given combination of dyes or method of procedure will best fit all cases; it is, indeed, more than probable that the compound stain will have to be modified somewhat as to its two components, depending on the source of the dyes.

After the stain, therefore, has been made up according to formula, it will be necessary to test it out on samples of sulphite and sulphate fibres. To do this, samples of unbleached sulphite and sulphate pulps should be prepared, and a few fibres of each placed on a slide, care being taken not to get the two samples mixed. The fibres are then dried, and stained as directed below, and then examined under the microscope. All the sulphate fibres should have a blue or blue-green colour, and all the sulphite fibres should have a purple or pink colour. If any purple fibres appear in the sulphate pulp this indicates that too much fuchsin is present in the combination, and a little more malachite green solution must be added to counteract this effect. If, on the other hand, some of the sulphite fibres show green or blue, there is too much malachite green in the combination, and more fuchsin solution must be added. Of course, the analyst must

be sure that he is using authentic samples of the two pulps for this test. When tested out in this manner and the proper combination found, the stain is ready to be used on unknown combinations of fibres containing either unbleached sulphite or sulphate, or both.

The stain should not be used for more than a few hours after being compounded and should be made up anew at least each day.

The method of preparing the samples of pulps or papers for staining, and of applying the stain, is as follows:

The sample is boiled in water, or in 0.5% aqueous solution of sodium hydroxide, for a few minutes, and the fibres thoroughly disintegrated by shaking in a test-tube, or other receptacle, about half filled with water, glass beads being added if the fibres cannot otherwise be separated. Several fibres are then removed by means of a teasing needle or, preferably, by means of a glass tube about  $\frac{7}{32}$  in. in diameter, placed on a microscope slide, and dried by the use of hard filter or blotting paper. Two or three drops of the compound stain are then placed on the fibres by means of a suitable dropper, or pipette, and allowed to remain 2 minutes, during which time the fibres are being teased apart and moved about in the stain on the slide. This teasing is necessary in order that the stain may have equal opportunity to act on all the fibres. At the end of 2 minutes the excess stain is removed with three or four thicknesses of hard filter paper, and the fibres treated with 3 or 4 drops of a weak aqueous solution of hydrochloric acid, made by adding 1 c. c. of concentrated acid (sp. gr. 1.19; HCl 37%) to 1 litre of distilled water. The acid solution is allowed to remain on the slide for from 10 to 30 seconds, during which time the fibres are teased and moved about rapidly. Following this, the excess acid solution is removed with filter paper, 3 or 4 drops of distilled water applied, the fibres quickly teased about, and the water absorbed with filter paper. If all the excess stain has been removed from the slide at this point, a drop or two of water may be added, the fibres spread about on the slide, and a cover-glass placed over them. But if too much stain remains on the slide at this point, it will be necessary to rinse again with distilled water before applying the cover-glass. After the cover glass has been placed in position, the fibres are ready for examination under the microscope.

**Other Microscopic Tests.**—The microscope offers practically the only means of examining many of the impurities which find their way into paper. Cinders and coal may be distinguished by their

structural differences; hairs or coarse fibres which cause breaks on the printing presses may be identified; sand from the grinders, rubber from the rag stock, bits of iron or brass, rosin spots, slime spots, bits of wood—all these may be picked out by means of the microscope, together with the proper stains and reagents. Each particular case is a miniature research, and no general directions can be given as conditions are different in every case.

Another way in which the microscope is of service is in the examination of the surface of the paper, particularly coated papers. In some cases it is possible to predict how the paper will print after making such an examination. It is also useful in examining defects, and in studying the way the half-tone dot takes on the surface in printing.

### PHYSICAL TESTS

**Machine Direction.**—There are several possible methods for determining the direction in which the paper was run on the machine.

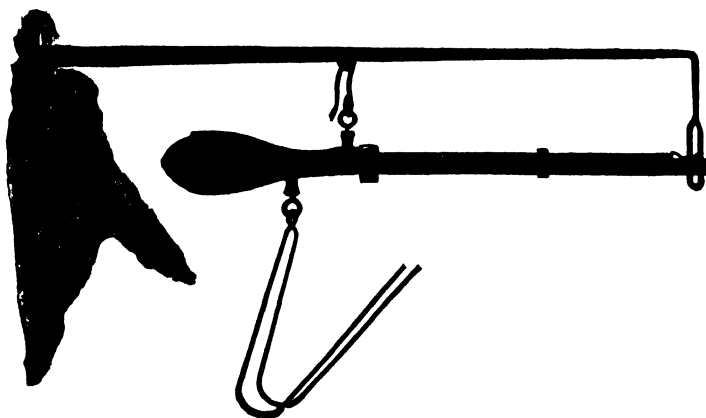


FIG. 88.—Beam type of paper scale.

The formation noted on looking through the sheet is often conclusive to the experienced observer; or the direction of the long diagonal of the wire marks, if the latter are at all prominent, will indicate the machine direction.

For well sized papers a circular piece may be cut out and wet with water on one side; it will at once tend to curl up into a cylinder whose axis corresponds with the machine direction of the paper.

Unsize paper may be made to respond to this test by first sizing with an alcoholic solution of rosin, or with an aqueous solution of gelatin, and then drying.

If two narrow strips are cut, one with and the other across the grain, and these are then placed together and held upright by their

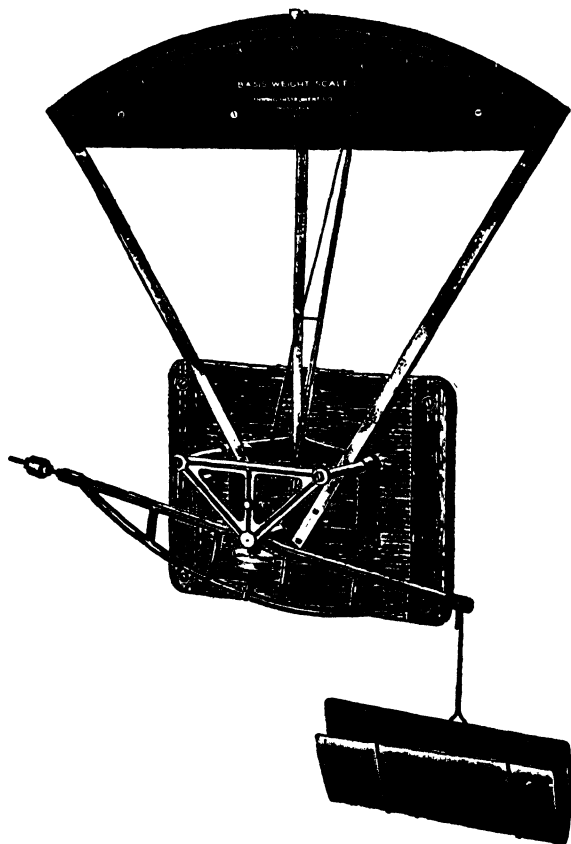


FIG. 89.

lower ends they will tend to bend over of their own weight. If the two strips cling together closely, the under strip is cut lengthwise of the sheet but if the under strip bends over more than the upper the latter was cut in the machine direction.

**Wire or Felt Side.**—This may often be determined by inspection, but in some cases the two sides are so much alike that it is hard to

tell them apart. Plunging the sample into water for a moment or two, and then blotting off the excess, will sometimes cause the wire marks to stand out plainly, where they were indistinguishable before. This method will often show the wire marks in coated papers.

**Weight of Sample.**—If a full size sheet of paper is available the weight per ream may be found on any one of several types of scale. The beam type shown in Fig. 88 is very commonly used in mill work; the folded sheet is placed on the hooks and balanced by the sliding

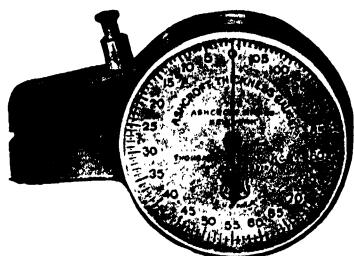


FIG. 90.—Ashcroft thickness gauge.

poise on the graduated beam. The position of the poise then indicates the weight in pounds per ream of either 480 or 500 sheets.

A scale of the quadrant type is shown in Fig. 89. This is designed to give the weight per ream quickly and without the use of any movable weights.

If the available sample is very small, a piece of measured area should be cut out and weighed on a chemical balance. The ream weight may then be calculated from the following formula:

$$\frac{(\text{Wt. in grm.}) \times 1.103 \times (\text{area of trade size desired})}{\text{Area of sample in sq. in.}}$$

$$= \text{Weight in pounds per 500 sheets of trade size desired.}$$

**Thickness.**—One of the many forms of spring micrometer offers a ready means for measuring the thickness of a sheet of paper. Figure 90 shows such an instrument, the dial of which is graduated in thousandths of an inch. The limit of accuracy of such an instrument is about 0.0005 in. and it should not be read closer than this.

An ordinary micrometer caliper may also be used to measure the thickness of paper but the pressure applied is not easily regulated unless the micrometer is of the ratchet type which prevents any excessive pressure being used.

It is well to test all thickness gauges from time to time by means of standard sheet-metal leaf gauges ranging from 0.001 to 0.015 in. This precaution insures the accuracy of the observations made on paper samples.

**Bulk.**—The “bulk” of paper, a factor which must be taken into consideration when planning a book of a definite thickness, is

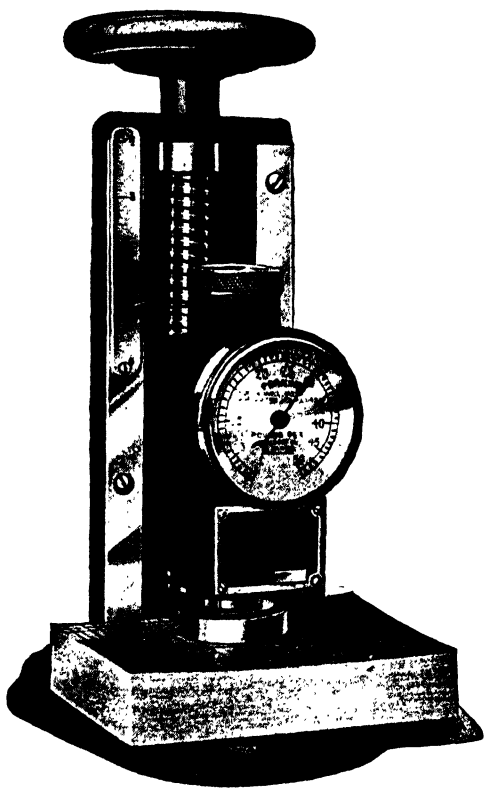


FIG. 91.—Pressure bulker.

generally expressed as the number of pages per inch in thickness. It is usually measured by making a “dummy,” or a pile of the required number of strips, and then measuring its thickness by means of a graduated sliding clamp. The pressure used in making this test may be either heavy, medium or light and this introduces an element of uncertainty into the results, even when the pressure

is specified, for no two persons would be likely to judge the pressure exactly alike.

The "pressure-bulker," Fig. 91, made by B. F. Perkins & Sons, has been designed to eliminate this source of error. When using this instrument the thickness of the dummy under any pressure is

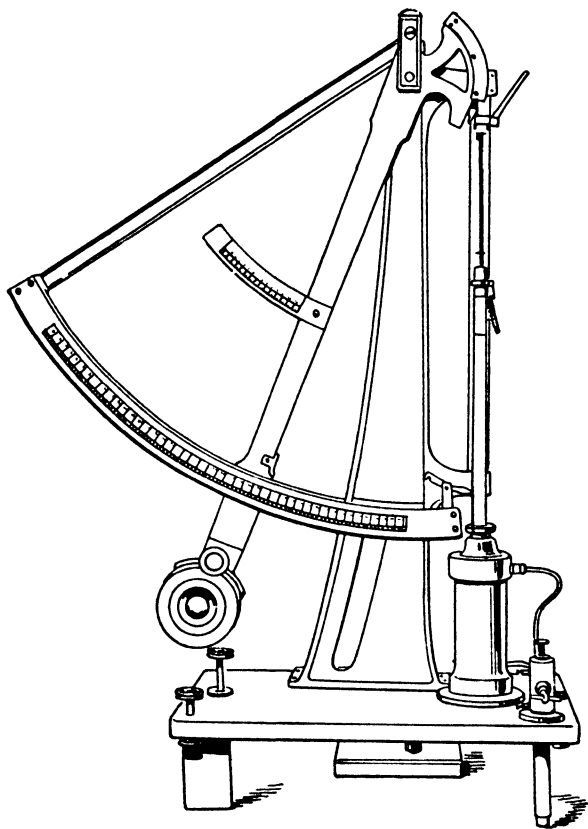


FIG. 92.—Schopper tensile machine.

indicated on the scale at the side, whilst the corresponding pressure applied to the dummy is read off on the circular dial.

**Bursting Strength.**—Two general types of apparatus are used for determining the bursting strength of paper. In the Mullen, Fig. 94, and the District of Columbia paper tester, the sample to be tested is clamped firmly against a rubber diaphragm. Hydraulic pressure

is applied to the under side of the diaphragm, which is thus forced through the paper. The dial indicates in pounds per square inch the pressure required to burst the paper.

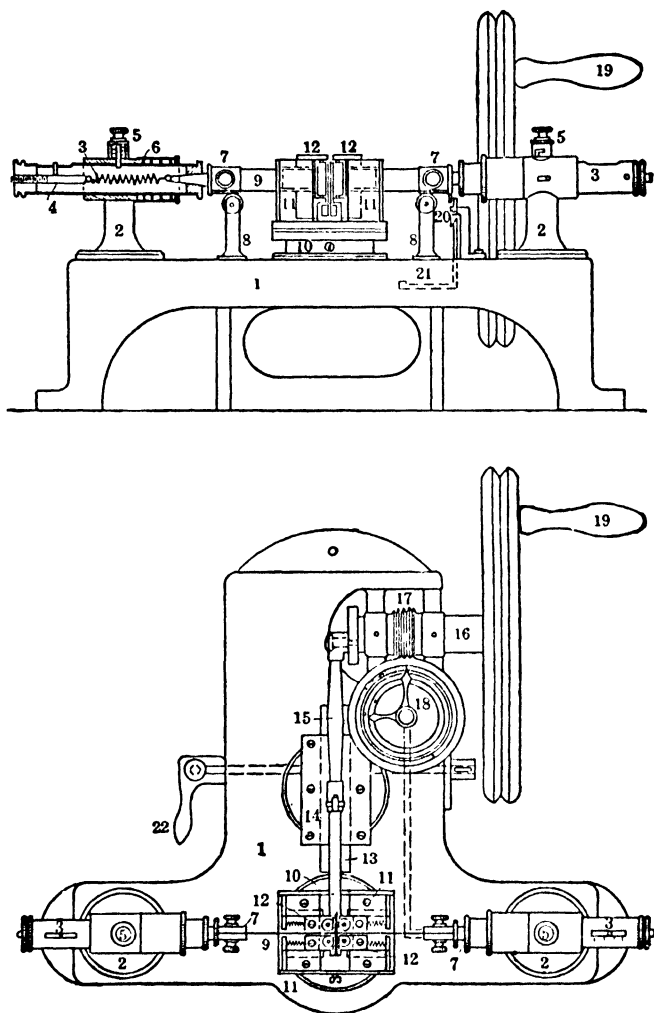


FIG. 93.—Schopper folding machine.

The Ashcroft tester, Fig. 95, is the only representative of the second type of instrument. The paper is clamped between two annular rings and then forced down onto a metal plunger supported

upon a spring. The pressure required to force the plunger through the paper is recorded on the dial in pounds per square inch.

To be of the greatest value the bursting strength must take into consideration the weight of the sheet tested. If the same standard size of sheet is used in each case, the ratio of strength to weight of

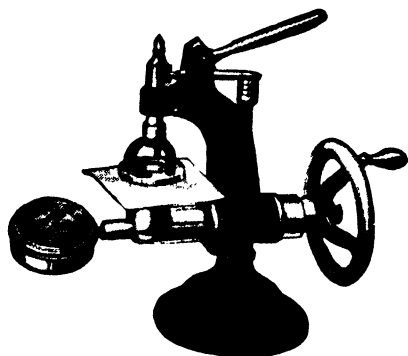


FIG. 94.—Mullen tester.

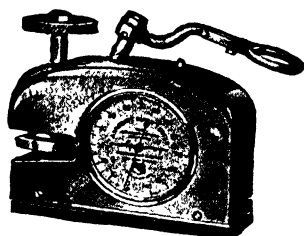


FIG. 95.—Ashcroft tester.

any two papers may then be directly compared. The strength ratio may be expressed as a percentage by the following formula:

$$\text{Strength ratio} = \frac{\text{Bursting strength} \times 100}{\text{Weight in pounds (on a size } 25 \times 40 = 500)}$$

**Tensile Strength.**—The most widely known instrument for determining the tensile strength of paper is the Schopper tester. The Perkins tensile tester is shown in Fig. 96.

A strip of paper 15 mm. wide by 180 mm. long is securely clamped at each end and, by moving the clamps apart, the paper is put under tension until it breaks. The motive force may be hydraulic pressure, as in the machine illustrated, or a hand wheel may be substituted to pull down the lower clamp. The force in kilograms applied to the paper is read off from an indicator which travels over the scale shown. The tensile strength in kilograms may be converted to the basis of pounds by the following formula:

Tensile strength in pounds per 1 in. width =  $3.73 \times$  (Tensile strength in kilograms per 15 mm. width).

It is customary to express the tensile strength of a paper as its "breaking length," which is the length of a strip which, if suspended at one end, would just break of its own weight. The breaking length may be calculated by the following formula:

Breaking length in yards =

$$\frac{(\text{Tensile strength per 1 in. width}) \times (13,889)}{(\text{Weight of sheet } 25 \times 40 - 500)}$$

With the Marshall paper tester, Fig. 97, which is much used in England, the width of the strip may be from 0.25 in. to 2 in., and the length from 2 to 12 in. The paper is fixed in a horizontal position, and the tension obtained by means of a spring.

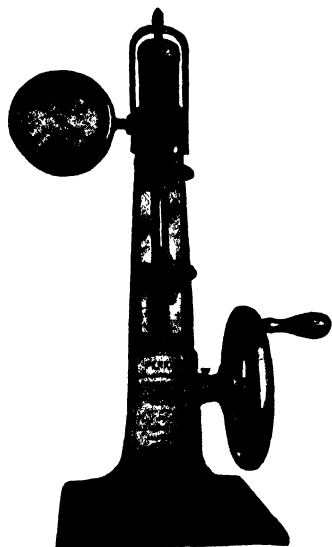


FIG. 96.—Tensile tester

Whatever instrument is used for determining the tensile strength, it is important that the paper be tested in both directions, with and across the grain, in order to obtain a satisfactory knowledge of its properties. Because of variations in the sheet, it is also necessary to take the average of at least five, and better ten, tests in each direction.

**Stretch.**—In addition to the tensile strength, the Schopper tester also shows the stretch at the instant of rupture. This is read off on a separate scale and is given directly as a percentage of the original length of paper between the clamps when the test was started.

**Folding Endurance.**—The only available instrument for measuring the folding endurance of paper is the Schopper folding machine, shown diagrammatically in front view and from above in Fig. 93. To make the test, a strip of paper 15 mm. wide and 100 mm. in length is clamped between the jaws (7) while the slot in the thin metallic plate fastened to the end of the shaft (13) is exactly in line with them. Tension is then applied to the paper by pulling out the shafts (4) which are connected with the jaws by the intermediate springs (3). By turning the wheel (19) a reciprocating motion is given to the shaft (13), and the slotted plate causes the paper to be folded back and forth. The rollers (12) serve to prevent friction of the paper so that the only wear comes in the crease caused by the slotted plate. For each revolution of the driving wheel the jaws are twice pulled

out to the maximum distance apart and the tension on the paper is then approximately 1000 grms. The minimum tension of about 730 grms. is applied to the paper when the slot in the metallic plate is in line with the jaws. These tensions are probably reduced somewhat because of the slight stretching of the paper. The repeated folding of the paper weakens it in the crease and it is finally broken

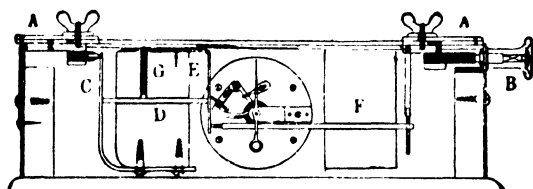


FIG. 97.—Marshall's paper testing machine (section).

by the tension. The dial (18) automatically indicates the number of double folds required to break the paper.

This test is one which is very greatly affected by the relative humidity of the atmosphere at the time the test is made. If accurate comparative results are wanted care must be taken that all tests are made at the same relative humidity. If possible it is best to make all physical tests on paper in a constant humidity room.

If desired the results of this test may be expressed as a folding factor as follows:

$$\text{Folding factor} = \frac{\text{Folding endurance}}{\text{Weight } 25 \times 40 - 500}$$

If the Schopper folder is not available, comparative tests may be made by folding strips back and forth for a definite number of times in a standard manner, and then determining the tensile strength. The difference between this test and that on the original paper shows the loss in strength due to folding.

**Tearing Test.**—A number of methods for determining the tearing strength of paper have been proposed, but as yet no standard method has been established. Of the instruments specially designed for this purpose the Elmendorf tearing tester, shown in Fig. 98, appears to be one of the best.

This instrument is based on the fact that the force required to tear the paper is measured indirectly by the work done in tearing the sheet. The paper is cut in strips  $2\frac{1}{2}$  in. in width and after swing-

If this apparatus is not available, a comparison may be made with a home-made apparatus. Cut a small, sharp-edged opening in a piece of cardboard and place this over a source of strong light. Over this opening place pieces of the paper to be tested until the light spot is completely obliterated. The number of sheets required is a measure of the opacity of the sample, and by determining the thickness per sheet it may be expressed as the absolute thickness of paper necessary to obliterate the light. This test should, of course, be made in a dark room.

**Gloss or Glaze.**—The Ingersoll glarimeter (L. R. Ingersoll, *Electrical World*, 1914, 63, 645), Fig. 99, offers a ready means for determining the gloss of a paper. This depends on the fact that light specularly reflected from paper at an angle of 57.5 degrees is almost completely plane-polarized; hence a determination of the amount of light thus reflected gives a measure of the gloss of the sample. The source of light is a tungsten lamp placed in one end of the box; the light from this falls on the sample, which is placed upon the block shown, and is reflected at the proper angle through a diaphragm, which has a small Nicol prism covering part of it and a smoked glass over the remainder to absorb about half of the light. At the other end of the tube is another Nicol prism mounted in a graduated circle which may be rotated, and which reads, with the aid of a vernier, to 5' of arc. On observing the light reflected, through the eyepiece, a divided field is seen, and by turning the divided circle the two parts of the field are matched so that the dividing line disappears. From the average of a number of such observations the gloss may be found from a table supplied with the instrument.

When working with colored papers a light red stained glass may be placed between the eyepiece and the eye of the observer. This eliminates color differences and permits the fields to be matched quite easily.

**Degree of Sizing.**—For papers which are not ordinarily very hard sized, such as book and magazine papers, a satisfactory test may be made by floating small pieces of the paper on a bath of writing ink and noting the time required for the ink to strike through to the upper surface. The ink used should be of a standard grade which can be readily obtained and always contain the same amount of free acid. It should be used only once and not returned to the bottle. The temperature of the ink is also very important; a standard

temperature should be selected and the ink maintained at that point for all tests. A variation of more than half a degree should not be tolerated. The papers to be tested should be exposed to the same atmospheric conditions for at least half an hour before placing on the ink, and the tests should be made by the same observer, as it is largely a question of personal judgment as to when the ink is through.

Several attempts to eliminate the personal factor by employing conductivity measurement have been made, and such methods are said to be in successful operation in at least one mill for making routine tests. They appear to be open to the objection that the electrolyte penetrates the point of weakest sizing, and the result therefore, indicates the minimum sizing of the sample, rather than the average.

For high-grade papers, as bonds, surface sized writings, etc., the flotation test does not give a satisfactory idea of the degree of sizing. For such papers Sammet (*Bureau of Chem. Circular No. 107*, also *Paper 10*, 1913, 15) recommends drawing a strip of paper over the surface of an iron tannate ink, draining and allowing it to dry naturally. When the surface is examined by means of a low power microscope a well sized paper will show no indication of absorption of the ink by the fibres. Any lack of uniformity of sizing will be indicated by variation in depth of colour of the surface.

Erasing the surface of the paper, preferably with a spun glass eraser, and again drawing it over the ink will give some idea of how far the sizing penetrates; a well sized sheet should show almost no additional ink absorption at the erased spot.

A satisfactory ink for these tests may be made as follows:

Tannic acid (dry).....	23.4 grms.
Gallic acid (crystals).....	7.7 grms.
Ferrous sulphate.....	30.0 grms.
Dilute hydrochloric acid (U.S.P.).....	25.0 c.c.
Phenol.....	1.0 grms.
Blue dye (Bavarian Blue S and J, No. 478).....	2.2 grms.
Water to make up to 1000 c.c. Allow to settle and decant from any sediment.	

**Volumetric Composition.**—It is sometimes desirable to calculate the volume composition of a paper. From the thickness of the sample and the weight of a given area the weight of a c.c. is calculated. By the usual analytical methods the percentage by weight

of the various constituents, as clay, fibres, size, etc., is found, and the weight of each in a c.c. is then calculated. Dividing the weight of each substance by its sp. gr. gives the volume which it occupies and the sum of all these volumes deducted from 1.0 gives the volume of air per c.c. of paper. Where only fibres, clay and rosin are present the results obtained by this method are fairly accurate, but the addition of other substances, such as are present in coated papers, renders the results much less reliable.

The volume of air in paper may also be obtained by determining the actual sp. gr. by first weighing in air and then in oil of known density. It is necessary to soak the paper in oil and submit it to reduced pressure for some time, to make sure that all air is replaced by oil.

**Air Permeability or Porosity.**—There is at present no simple and accurate apparatus for measuring this property of paper. Measurements have been made by Herzberg (*Mitt. k. Materialprüf.*, 1915, 33, 142–143), who passed air through a definite area of the paper and measured the amount by a gas meter provided with special devices to render it more accurate and sensitive. Equipment of this sort is not generally available.

It has been proposed by Seiter (*Chemist-Analyst* No. 21, April, 1917) to test waxed or waterproofed papers by using them as diaphragms in a dialysing apparatus. If ferric chloride is placed above the paper and potassium ferrocyanide below, the time required for the formation of Prussian blue will indicate the porosity of the paper.

**Grease-proof Properties.**—The best test for the grease-proof properties of paper is to lay the sample on white paper, pour on to it a little oil of turpentine and spread it around with a little absorbent cotton. If any stain develops on the white paper, the sample is not grease-proof.

A rough test which may be applied to imitation parchment is to heat the sample for a moment over a small flame. If the surface is impervious, blisters will be formed, whilst pin holes will prevent their formation. This test is reliable in most cases, but is not absolutely conclusive, as some papers which blister are not entirely grease-proof, while others which do not blister have proved satisfactory in use.

## CHEMICAL TESTS

**Moisture.**—The moisture in most papers may be determined by weighing a sample in a stoppered weighing bottle, drying at  $100^{\circ}$  to  $105^{\circ}$ , cooling in a desiccator, after replacing the stopper, and again weighing. The loss in weight represents moisture. The dried sample should never be weighed in the open air, if any degree of accuracy is expected, because of the great rapidity with which dry paper absorbs moisture from the surrounding atmosphere.

If substances are present which lose water of crystallization or of constitution at  $100^{\circ}$ , this procedure gives inaccurate results. The presence of calcium sulphate, either as a filler or as satin white in coated paper, is one of the chief reasons for this error, since three-fourths of its water of crystallization is driven off at the temperature named, and this loss makes the hygroscopic moisture appear entirely too high. In such cases the sample may be dried to constant weight in a desiccator over sulphuric acid, or the amount of calcium sulphate may be determined by chemical analysis, and the moisture which it would lose on drying deducted from that found by the method of direct drying.

**Ash.**—The ash in paper may be determined by igniting a weighed sample of the paper in a tared dish, cooling in a desiccator and again weighing the dish and ash. If the sample taken is exactly 1 grm. in weight, the weight of ash found may be converted to per cent. by moving the decimal point two places to the right. The heating may be done over a burner or in a muffle furnace; if the former is used, the carbon will burn off more quickly if a piece of platinum foil, bent in the form of an arch, is placed over the dish to reflect the heat downward onto the sample. The ignition may be made in porcelain, silica, platinum or any material not changed in weight by heating to a bright red heat. Platinum, or some of its substitutes, will probably be found cheapest in the long run, in spite of its high first cost, especially if the ash is to be weighed in the dish in which the ignition is made. The advantages of platinum are its constancy of weight, and the fact that both ignition and cooling are more rapid than in the case of either silica or porcelain. A flat, shallow dish will be found to give more rapid combustion than a deep dish or crucible.

For ordinary work, the air-dry paper is used and the sample need not be weighed closer than 0.005 grm.; for work of extreme accuracy, the sample should be weighed in a closed weighing bottle after drying at 100°. Care must be taken that the ash is not blown out of the dish by convection currents, and that no loss occurs from the sudden rush of air into the desiccator when the cover is removed. The use of a desiccator may be avoided by cooling the dish slightly, and then pouring the ash into a counterpoised aluminum pan as soon as danger from loss by air currents is over. When thus handled the ash cools almost instantly and may be weighed at once.

The ash, as thus determined, includes all incombustible and non-volatile matter in the paper. It may be derived from several sources, loading or filling materials, substances used in the coating, mineral colouring matters, ash due to size and alum, or materials in the fibres employed. The ash in paper may amount to as much as 5% due to materials other than fillers, but if this figure is exceeded, it is safe to say that some loading material has been added.

In order to calculate the amount of filler from the ash present, it is necessary to know the ash in the fibres used and the loss in weight which the original filler suffers when it is ignited. The ash in fibrous raw materials is given by Wrede (*Paper*, Jan. 31, 1912) as follows:

MATERIALS	PERCENTAGE, ASH
Bleached linen half stuff.....	0.12-1.86
Bleached cotton half stuff.....	0.24-0.79
Unbleached cotton half stuff.....	0.24-1.12
Sulphite, unbleached.....	0.48-1.25
Soda.....	0.36-1.40
Adansonia.....	5.77-7.19

The loss in weight of air-dry fillers may be as low as 1% for some of the ground minerals, or as high as 21% for crystallized calcium sulphate. An average figure for air-dry clay would be about 12%.

The nature of the material used as a filler may be determined in many cases by making a chemical analysis of the ash. The methods followed are those given in the numerous text books on analytical chemistry to which reference should be made.

**Paraffin.**—The amount of paraffin wax or similar substance present in a paper may be determined by extracting a weighed sample with gasoline or carbon tetrachloride in a Soxhlet apparatus or an Erlenmeyer flask fitted with a reflux condenser. The sample taken should be large enough to give a weighable amount of paraffin wax

and if the Erlenmeyer flask is used it will probably be necessary to extract a second time with a fresh quantity of solvent. When extraction is completed the solvent is evaporated and the residue dried and weighed. Sometimes the paraffin tends to creep over the sides of the dish; in this case it is better to weigh the paper after extraction and consider the loss in weight as paraffin wax.

From the standpoint of solvent power, either gasoline, carbon tetrachloride<sup>1</sup> or chloroform is satisfactory, but the tetrachloride is not inflammable like gasoline nor does it produce anaesthesia so readily as chloroform.

**Rosin.**—There is no qualitative test for rosin which is absolutely reliable in all cases and it is therefore necessary to form an opinion from the indications of a number of different tests. When a little ether is poured onto the surface of a paper and allowed to evaporate it will first dissolve any rosin present and then deposit it as a ring surrounding the part which was wet with the ether. Any other substances which are soluble in ether will also give this ring.

If a sample of the paper is boiled with 5 c.c. of acetic anhydride in a dry test-tube, cooled, and a little concentrated sulphuric acid poured carefully down the side of the tube the presence of rosin will be indicated by the formation of a pink ring.

A sample of the paper may be boiled for a few minutes in a little glacial acetic acid, which is then poured into a little distilled water. If rosin is present, this will cause a decided turbidity, but other substances may cause a slight opalescence, and such a result must not be taken as proving the presence of rosin.

If a drop of concentrated sulphuric acid and a grain or two of sugar are placed on a rosin sized paper, a distinct raspberry-red colour will develop, whilst if the paper is unsized, a brownish colour with only a trace of pink appears. Albuminous materials also give this test, and they must be proved absent before the test can be taken as indicating rosin.

The quantitative estimation of rosin is best carried out according to the method of Sammet (*J. Ind. Eng. Chem.*, 1913, 5, 372). 5 grms. of the paper are cut into strips about 0.5 in. wide and folded into many small crosswise folds. These are placed in a Soxhlet extractor and extracted with acidified 83% alcohol made by mixing 100 c.c. of 95% alcohol and 15 c.c. of acidified water (100 c.c. of

<sup>1</sup>There have been several deaths in England with anaesthesia through inhalation of  $\text{CCl}_4$ .

distilled water and 5 c.c. of glacial acetic acid). The flask of the extraction apparatus should be placed directly in boiling water, and the extraction continued until siphoning has taken place from 6 to 12 times, according to the kind of paper. Remove the extract to a beaker and evaporate to a few c.c., on a steam bath. Cool, dissolve in 25 c.c. of ether and transfer to a 300 c.c. separating funnel to which has been added about 150 c.c. of distilled water and a little sodium chloride to prevent emulsification. Shake, allow the ether layer to separate, draw off the water into a second separating funnel and again extract with a second portion of ether. The combined ether extracts are washed twice with 100 c.c. portions of water, or until the line between ether and water is distinct and sharp. Transfer the washed extract to a weighed dish, evaporate the ether very cautiously to avoid loss, dry at  $98^{\circ}$  to  $100^{\circ}$  for exactly 1 hour, cool and weigh.

**Glue and Casein.**—There is no satisfactory method for estimating either one of these two materials when the other is present, for both are nitrogenous in character.

The presence of casein in any appreciable amount may be detected by moistening the paper with Millon's reagent and warming gently over a flame or a steam jet. This gives a brick-red colour when casein is present. Coated papers which contain much satin white should first be treated with a little dilute nitric acid, to neutralize the alkali in the satin white and prevent the formation of a yellow colour.

Glue or gelatin may be detected by boiling the paper in a little water, filtering if necessary, and adding a little tannic acid solution. This causes a greyish, flocculent precipitate if glue is present. The test is also given by any casein which may dissolve and the precipitation is prevented by the presence of starch, so that in the presence of either of these materials there is apparently no means of proving glue to be either present or absent.

The Kjeldahl method offers practically the only means for determining the amount of glue or casein in paper, and it is reliable only when they are not present together. Weigh out from 3 to 5 gm. of the paper, cut into small pieces and place in a Kjeldahl digestion flask together with 10 gm. potassium sulphate, 0.7 gm. mercury, and 25 c.c. of concentrated sulphuric acid. Place the flask in an inclined position in a hood with a good draft and heat cautiously until strong frothing ceases. Increase the heat until the acid boils and keep it at

this point until the solution becomes colourless or pale yellow. Allow the flask to cool and add to its contents 30 c.c. of a 4% potassium sulphide solution. Pour carefully into the flask, so that it will mix as little as possible, 50 c.c. of sodium hydroxide solution (saturated solution) or enough to give a strongly alkaline reaction; connect the flask with a condenser, mix the contents of the flask by shaking and distil until all ammonia has been driven off. The distillate must be collected in a known amount of standard acid in such a way that no ammonia is lost, and when the distillation is completed the excess of acid is determined by titration with standard alkali, with the use of Methyl Red or sodium alizarine sulphonate as indicator.

To be sure of the purity of the reagents used a blank test should be made in the same way, using the same amounts of reagents, but omitting the paper.

The percentage of nitrogen found by this method may be multiplied by 6.3 to convert it to casein, or by 5.6 to convert it to glue. As the percentage of nitrogen in casein or glue varies with different lots, this method gives only an approximate or average result, unless it is possible to determine the nitrogen in the casein or glue actually used in making the paper under examination.

**Starch.**—The presence of starch in paper, in any appreciable amount, is readily detected by moistening it with a dilute solution of iodine in potassium iodide. This develops a blue colour, the intensity of which varies with the amount of starch present. Hydrocellulose, when brought into direct contact with iodine solution, will also give a blue colour, so it is well to confirm the test by boiling a little of the paper in water, cooling the solution and testing with a drop or two of iodine solution. Hydrocellulose, being comparatively insoluble in water, will not interfere with this test.

After staining with iodine solution microscopic examination will show whether the starch was used without cooking, or whether the granules have been burst by boiling. When the paper is torn so that it splits on the edge before wetting with iodine solution it is often possible to tell whether it was sized only on the surface, or whether the starch is present throughout the paper. In the former case only the surface will be coloured, whilst if the starch was used in the beater, it will be uniformly distributed among the fibres. When much starch is used in the beater, part of it is cooked in passing over the driers and this is drawn to the surface by capillary action and

evaporation, so that the paper has the appearance of being surface sized. Under such circumstances it is not possible to determine with certainty whether the paper is surface sized or not.

The quantitative estimation of starch in paper may be made with a reasonable degree of accuracy by the method of Voorhees and Kamm (*Paper*, 24, 1919, 1901), provided the fibres used consist principally of sulphite and rag stock. If much soda fibre or groundwood is present, an accurate test is out of the question, because materials are extracted from these fibres which react with Fehling's solution and in consequence give too high results.

The solutions employed are a 50% solution of acetic acid, a 10% solution of potassium ferrocyanide and the usual Fehling's solution. For the latter the copper sulphate and Rochelle salt should be dissolved and preserved separately, and the solutions mixed in equal volumes only when they are to be used. The Fehling's solution may conveniently be standardized against one of the starches commonly used in paper making, hydrolysing and titrating under the same conditions as used for the actual analysis of the paper. The procedure for analysis is as follows:

The paper to be analysed is tested with the usual iodine reagent. If but a trace of starch is present, no acetic acid is required in extraction. A 5 grms. sample is cut into small pieces and placed in a 500 c.c. round-bottom flask. 200 c.c. of water are added and 5 c.c. of glacial acetic acid are run in, making a 2.5% solution. The flask is connected with a reflux condenser by means of a clean rubber stopper and the contents boiled vigorously for 1.5 hours. The extract is decanted through a Büchner funnel equipped for suction filtration, and the pulp washed with about 50 c.c. of hot water. To the filtrate are added 15 c.c. of HCl (37%) and boiling continued for 30 minutes, the volume of the solution being permitted to decrease by evaporation to about 200 c.c. The hot acid solution is neutralized by the addition of solid sodium carbonate until effervescence ceases and the volume is determined. This solution is titrated into a measured quantity of Fehling's solution (2 to 10 c.c. according to the amount of starch present). After each addition of sugar solution the mixture is heated to the boiling point, and maintained at that temperature for 1 minute. The reaction mixture may be diluted, if this is considered desirable. The end point is determined on a spot-plate with a potassium ferrocyanide-acetic acid solution, and is that point at

which no immediate colour is produced on the plate; it may be determined to within 0.5 to 1 c.c. of the sugar solution, depending on the volume of solution employed. It was found that the potassium ferrocyanide became coloured when allowed to remain a number of days with the acetic acid, and that sharper and more distinct end points can be obtained if the acid is added separately to the spot-plate when the test is to be made. One drop of each solution is used for a test.

**Free Acid.**—The free acid in paper may be determined as follows: Tear a 10 grm. sample into small pieces, place in a porcelain casserole, cover with a little water and heat gently for 1 hour over a water-bath. Pour off the solution and wash the paper with a little water, adding the washings to the solution. Make up to 100 c.c., add 2 drops of litmus solution and pour into a 100 c.c. Nessler tube (long form). In a similar tube place 100 c.c. of distilled water to which two drops of litmus solution have been added. To the first tube add N/10 sodium hydroxide solution until the colour of the two tubes is the same. The acidity may be expressed as per cent.  $\text{SO}_3$ .

**Amount of Coating.**—This may usually be determined by weighing a sample of known area, soaking it for a few minutes in warm, dilute ammonia solution and then brushing off the coating with a camel's hair brush. After removal of the coating the paper is air-dried and again weighed, the loss in weight being recorded as coating. If the coating is difficult to remove in this manner, the use of stronger ammonia sometimes helps, but there are occasional samples which are so thoroughly waterproofed that the coating is absolutely unaffected by this treatment. In such cases it is impossible to determine the amount of coating.

There is quite a large personal factor in this test, as it is often a question whether it introduces a greater error to leave traces of coating on the paper or to lose a little of the fibre in trying to remove the last bit of coating.

**Sulphur.**—This test is demanded by the users of tissue papers for wrapping silver ware. The method, which depends on the stain produced on lead acetate paper, is as follows (Sutermester, *Pulp and Paper Mag. Can.*, 1917, 15, 1021).

The apparatus consists of a 500 c.c. round-bottom flask with a neck about 2 in. long and 1 in. in diameter. The mouth of this neck is ground to a flat surface, and on this is placed a glass tube about 4 in.

long and 1 in. in diameter, the lower end of which is also ground flat to fit tightly upon the upper surface of the neck of the flask. The whole is so arranged that, after placing a piece of filter paper between the two ground surfaces, the tube and flask can be securely clamped together so that all gas generated in the flask must pass through the filter paper and then up through the superimposed glass tube.

The procedure for the testing of tissue papers is as follows: A sample of 25 sq. in. is taken and its weight determined. It is then shaken up in a wide-mouth, glass-stoppered bottle with 10 c.c. of distilled water; when partial disintegration has taken place, another 10 c.c. of water is added, and the shaking continued until the paper has been completely reduced to pulp. The larger part of the pulped mass is now transferred to the flask described above, and the residue which is left in the bottle is rinsed into the flask with a mixture of 10 c.c. of sulphur-free phosphoric acid and 20 c.c. of water.

Prepare turnings from the highest grade, pure stick zinc, which must be free from sulphur and arsenic. Treat 1 gm. of these turnings with 10 c.c. of a dilute solution of copper sulphate containing about 0.002 gm. actual copper. After a few minutes all the copper will have deposited and the turnings are then thoroughly washed to remove every trace of zinc sulphate.

The turnings are added to the flask and a wad of cotton inserted in its neck. Between the two ground glass surfaces is then clamped a piece of filter paper about 2 in. square which has been perforated with small pin holes about  $\frac{1}{8}$  in. apart and which just before use is moistened with several drops of lead acetate solution. Finally, a loose wad of cotton is placed in the tube above the paper.

The flask is placed on the steam bath and allowed to stay, with occasional shakings, for an hour. The filter paper is then removed from the neck of the flask and air-dried. It is best compared with the standard test pieces by placing them side by side on a piece of white paper and covering them with a thin piece of clear, white glass. The standard test pieces are prepared by using sulphur-free cotton in the flask, instead of the disintegrated paper, and adding to this definite volumes of a very weak solution of sodium thiosulphate whose strength is accurately known. The sulphur-free cotton is prepared by boiling absorbent cotton in weak caustic soda solution and washing thoroughly with distilled water.

The sensitiveness of this test is such that the presence of 0.000001 grm. of sulphur in the flask will give a distinct colour on the lead acetate paper. From tests of a considerable number of papers which have been found satisfactory in actual practice it has been proved that tissue paper is safe for wrapping silver ware if it does not contain more than 0.000002 grm. of sulphur per 25 sq. in. of paper (about 0.25 grm.).

When carrying out this procedure it is necessary to be sure that the sample has been kept away from dust and laboratory fumes, and that it has been handled as little as possible, and then only with fingers which are perfectly clean and free from perspiration. The purity of the zinc and phosphoric acid should be proved by careful blank tests, and no other acid should be substituted for phosphoric, unless it is first proved to be absolutely reliable. The standard test papers for comparison should be freshly made each time the test is carried out as they are not permanent even under the best conditions of storage.

**Unbleached Fibre.**—In addition to the microscopical methods already described, it is possible to employ chemical tests for detecting unbleached sulphite fibre. If the sheet under examination is moistened with Millon's reagent and warmed over a steam jet brownish hairs will appear if unbleached sulphite is present. The absence of these brownish fibres indicates that unbleached sulphite was not used. From the proportion of such fibres an approximate estimate of the amount of unbleached fibre used may be made, provided the amount was not too great. If the paper consists mostly of unbleached fibre, or if much ground wood is present, the entire surface becomes brown and no estimate of proportions can be made.

**Groundwood.**—Several stains are available for the detection of groundwood in paper without the use of the microscope, among them being aniline sulphate, paranitroaniline and phloroglucinol.

The aniline sulphate stain is prepared by dissolving 5 grm. of the sulphate in 50 c.c. of distilled water and adding 1 drop of concentrated sulphuric acid. This gives a yellow colour with groundwood, but the test is not so distinctive as that with phloroglucinol.

1 grm. of paranitroaniline dissolved in 405 c.c. of distilled water and 30.5 c.c. of sulphuric acid (sp. gr. 1.84) gives a very satisfactory testing solution. This gives quite an intense orange colour with groundwood and the colour develops without drying the sample.

Phloroglucinol is one of the best known reagents for detecting groundwood. This solution is prepared by dissolving 5 gm. of phloroglucinol in 125 c.c. of distilled water to which has been added 125 c.c. of concentrated hydrochloric acid. This solution gives a magenta colour with groundwood, and the depth of colour produced in a paper is, to a certain extent, an indication of the amount of groundwood present. A very light shade may be given by partly cooked jute, unbleached sulphite, and some other fibres, and is therefore not conclusive proof of the presence of groundwood. This stain should be kept in the dark in order to preserve its staining properties which are gradually lost on exposure to light.

A method for the quantitative estimation of groundwood by means of phloroglucinol has been worked out by Cross, Bevan and Briggs (*Papier Ztg.*, 1907, 32, 4113, 4479).

The solutions required are:

1. 2.5 gm. of pure phloroglucinol dissolved in 500 c.c. of hydrochloric acid, (sp. gr. 1.06).
2. 1 c.c. of 40% formaldehyde in 500 c.c. of hydrochloric acid (sp. gr. 1.06).

The sample of paper to be examined should be reduced to a loose powder by rasping, but it is not usually necessary to extract sizing matter. Dry 2 gm. of the powdered sample at 100°, weigh, transfer to a dry flask and add at once 40 c.c. of phloroglucinol solution. Stopper the flask, shake and allow it to stand overnight if possible, or at least several hours. Filter through a very little cotton placed in a funnel and measure out 10 c.c. for titration. Dilute this sample with 20 c.c. of hydrochloric acid (sp. gr. 1.06), warm to about 70° and add the formaldehyde solution from a burette in lots of 1 c.c. at a time. After each addition allow it to stand for 2 minutes and then test by removing a drop and placing it on a piece of partly sized newspaper. At the end of 10 seconds shake off the drop of liquid and note whether a red colour shows; if it does, add more formaldehyde, and when the reaction is nearly completed reduce the amount added each time to 0.25 c.c. When no red colour develops on the paper the reaction is completed; 1 part of phloroglucinol in 30,000 will produce a colour.

A blank test should be made with 10 c.c. of phloroglucinol solution, and the absorption of the groundwood calculated from the difference between the two titrations. It is very essential to use pure phloroglucinol and a considerable supply should be kept on hand so that

its quality may be assured. The temperature must be kept at 70 degrees whilst titrating, and the ratio of sample to phloroglucinol solution must be kept constant.

Different fibres have been found to have the following absorption values:

	PER CENT.
Cotton.....	0.20
Esparto.....	0.50
Sulphite, unbleached.....	0.90-1.03
Sulphite, bleached.....	0.90-1.00
Groundwood, brown.....	5.52
Groundwood.....	7.87-8.15

If the absorption values for groundwood and sulphite are assumed to be 8.0 and 1.0 respectively then

$$H = \frac{100 (P - 1.0)}{8.0 - 1.0}$$

Where

$H$  = per cent. of groundwood.

$P$  = absorption value of ash-free dry fibre in sample.

## WOOD PULP

A number of the tests described in the previous part of this chapter may be applied to wood pulps, either mechanical or chemical, as a means of grading them for quality; this applies particularly to microscopic examinations and to such chemical tests as ash, rosin, sulphur, etc. In addition, there are several other tests which will throw light on the suitability of the pulp for the use in question and finally there is the ever-present problem of moisture determinations.

**Testing Pulp for Moisture. Bales and Rolls.**—Since the purchase of wood pulp is based on a moisture content of 10% in so-called "air-dry pulp" it is obvious that accurate methods of sampling and testing are of prime importance. In spite of the great amount of attention which has been given this problem, there is still such diversity of opinion on the subject that a good-sized volume could be written in discussing all the proposed methods. The procedure finally adopted by the American Paper and Pulp Association and the Association of American Wood Pulp Importers as the official method for sampling and testing baled pulp is as follows:

"All tests must be made by a chemist duly authorized and approved by the joint committee representing the Association of American Wood Pulp Importers and the American Pulp and Paper Association on one side and the Scandinavian Wood Pulp Associations on the other side, and must be made strictly in accordance with the following instructions—otherwise the committee reserves the right to withdraw the approval of any chemist at any time.

"Before proceeding to the weighing and sampling the chemist must ascertain that not less than half of the parcel in question is available.

**"Number.**—Not less than 5%, nor more than 10% of the entire shipment, but not less than 10 bales shall be sampled. Samples to be drawn only from sound and intact bales, from different sections of the entire shipment, and analyst shall be careful to observe that no unusual conditions prevail in the selection of the bales. The accurate weight of all bales sampled to be ascertained by sworn weigher before sampling, or, wherever sworn weigher is not available, by a competent person who must make sworn affidavit that weights are correct, and no other bales than those weighed to be sampled, and whenever bales are numbered, the number is to be given in addition to the weight.

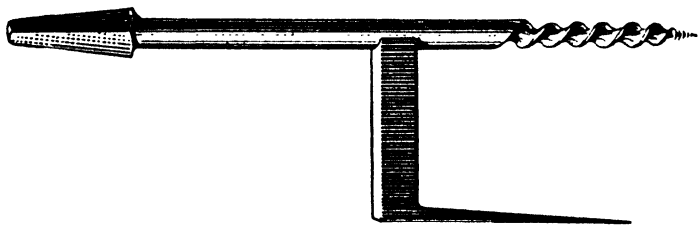


FIG. 100.—Sample cutter for boring bale pulp.

### Method of Sampling

**"Depth of Boring.**—The sample shall be taken by boring into a bale to a depth of 3 in. (7.62 cm.) with a special auger which cuts a disc about 4 in. (10.16 cm.) in diameter.

**"Selection of Discs.**—The discs shall be removed and ten of them taken as a sample, these to be selected as follows:

1 disc 2nd sheet from the wrapper.

2 discs 1 in. (2.5 cm.) deep.

3 discs 2 in. (5.05 cm.) deep.

4 discs 3 in. (7.62 cm.) deep.

10

**“Location of Borings.**—The holes to be bored shall be so located that in five successive bales they will represent a portion extending diagonally across the bale. Each bale to be bored but once. The first hole to be bored at the corner, the edges of the cut being at a distance of 1 in. from the edge of the bale. The second cut shall then be made half way between the location of the first cut and the centre of the bale, the third bale shall be cut at the centre, the fourth bale half way between the centre and the corner, and the fifth bale in the opposite corner in a position corresponding to the first.

“All samples must be either weighed immediately after being drawn from the bales by accurate scales, or, when this is impracticable, must be put into air-tight vessels, made of metal or glass, with ground-glass or metal stoppers, and due care must be used in the transportation of such samples until they can be properly weighed at the laboratory of the chemists. The entire bulk of samples selected from the bales must be dried out for the test. The temperature in the drying oven shall not exceed 100° (212° F.).

“Chemists must have proper and adequate equipment for weighing and sampling the bales, and for the weighing and drying of samples.

“All sampling of pulp must be done by or supervised by the approved chemist personally, or by his bona fide assistants—each chemist to file with the committee a complete list of his bona fide assistants who will do the sampling, such list to have the approval of the committee. The chemist will be held responsible for the correct sampling by his approved assistants. The committee shall at any time have the privilege of investigating the sampling done by chemists or their assistants.

“Every test certificate shall clearly state the name of the person who did the sampling.

“The test certificates hereafter shall be uniform and in accordance with forms to be approved by the committee, a sample draft of which will be furnished by the committee to each chemist.”

This method may also be used for dry pulp in rolls, making the first boring 1 in. from the end, the next half way between the end and the middle, the third at the middle, and so on.

This method possesses the advantage of speed and it also leaves the bales or rolls in good condition, which is a consideration of much importance where the sampling is done on the docks and the pulp has to be again loaded and shipped. Careful tests have demonstrated, however, that it favors the seller of the pulp, since the outside of the bale loses moisture more rapidly than the inner portion and its loss in weight does not keep pace with the decrease in the moisture test in the sample. This explains the reason why retests on bales which have been stored in a comparatively dry place for some time nearly always show a greater net weight of fibre than when first tested.

The quarter-sheet method, which is probably more accurate than the disc method, has the disadvantages that it requires much time and labour and leaves the bales in an unsatisfactory condition for storage or further shipment. In this method the bales are opened and a quarter of a sheet taken from various depths in the bale in such a way that the combined samples would cover its entire area. The number of samples taken from each bale varies with different workers from two to ten; in the latter case the first sample is taken from the second sheet from the wrapper and the others are equally spaced between the second sheet and the centre of the bale.

The wedge method differs from the quarter sheet in the shape of the sample which is in the form of a wedge with its apex at the centre of the sheet and its base at the edge. The samples are cut in rotation so that the combined sample represents the entire area of the bale. This method has the same advantages and defects as the quarter-sheet method.

**Lap Pulp.**—Methods for testing lap pulp are not so well standardized as for bales, though much attention has been given to the subject. The Technical Section of the Canadian Pulp and Paper Association have investigated the matter carefully (Slack, *Pulp Paper Mag. Can.*, 1919, 17, 265) and their tentative recommendation is that in the case of lap pulp from ordinary wet machines or Rogers wet machines a strip 3 in. wide should be cut clear across the sheet and to its full thickness. A sample of this size should be taken for each ton of wet fibre in the shipment.

For hydraulic pressed pulp Woodruff's (*Paper*, Oct. 3, 1917, 86) wedge method is to be recommended. In this method the samples are marked by a template having an angle of 9 degrees and at the apex a disc divided into 40 parts. This disc is placed at the centre of the lap and a pencil mark made along each side of the template. The next lap is marked in the same way, but after moving the wedge of the template to the second position on the disc; and so on until 40 laps are marked. They are then taken to a circular saw and the wedges sawed out. If necessary to reduce the size of the sample, the wedges may be split in half.

The drying of all samples for moisture should be done in an oven sufficiently large so that the pulp may be spread out thin. There should also be provision for the escape of moisture laden air. While the official method states that the temperature should not exceed 100° (212° F.), it is the writer's opinion that higher temperatures do no harm, and experiments have proved that even exposing the sample to 110° (230° F.) did not change its weight after it had been dried at 100°. All samples should be weighed in closed containers, especially after drying, and it is a wise precaution to replace the sample in the oven and give it a second drying to make sure that constant weight has been reached.

**Strength or Beating Test.**—This test was devised by the writer (*Paper*, Nov. 10, 1915) to show the variations in the strength developed in different sulphites by the same beating treatment. Several different methods of applying the test have been worked out, but the original procedure is about as follows:

After allowing the samples under examination to become air-dry two lots of 50 grm. each are weighed out. One of these is reduced to a pulp by first soaking in 1000 c.c. of water and then rubbing between the hands. This pulp is then rinsed into a small pebble mill with 1000 c.c. more water, the jar closed, and allowed to turn at 60 r.p.m. for exactly 1 hour. The entire contents of the jar are then turned onto a coarse mesh screen and the pulp washed off the stones into a pan beneath the screen. The pan is then filled up to a certain mark, and four sheets are made by means of a hand mould, two dips being taken for each sheet and the hand mould being reversed between dips to insure uniformity in the thickness of the sheet on the two edges. The sheets are pressed between felts in a copying press, air-dried, and then tested for strength with an Ashcroft

tester. The second sample of 50 grm. may be broken up to a pulp and made into sheets without beating to show the strength in comparison with the beaten stock.

In order to get concordant results from this method careful attention must be given to a number of factors. The amounts of fibre and water must be strictly adhered to, as slight variations cause appreciable changes in the bursting strength. The time of beating must also be carefully regulated and variations of more than 1 or 2 minutes should not be permitted.

The size of the pebble mill and the number and size of the pebbles have a considerable effect on the results, and two mills of the same make and rating may vary enough to give quite different results. When two mills are used it must be assumed that one is correct, and then the charge for the other adjusted by trial so that it gives the same results as the first.

One of the most important points is the moisture in the sample when it is prepared for testing. A wet pulp will not give the same test as it will after air-drying, as the drying out process changes the character of the fibre in such a way that beating does not develop so much strength. This is not a reversible reaction, and soaking dry fibre in water will not bring it back to its original condition before drying.

The hand mould for making sheets is open to certain objections when fibres of widely differing properties are treated, since the "slowness" of certain of the fibres makes the sheets thin and consequently weaker than they should be. This trouble may be overcome by using some form of sheet-making device in which a constant volume of stock is used so that the sheets are of practically constant weight regardless of how long the fibre has been beaten.

As originally applied, this test showed the strength developed under constant beating conditions. Another way of carrying it out is to beat a number of samples for different lengths of time; this will show the maximum strength obtainable with that particular fibre and also how long a treatment is necessary for its development.

The beating test has proved of much value in testing sulphite, soda and sulphate pulps, but is apparently not suitable for ground-wood as this does not hydrate under the beating treatment as do the chemical pulps.

**Colour Comparisons.**—The Committee on Sulphite Pulp of the Technical Association of the Pulp and Paper Industry have devised a method (*Paper*, Nov. 8, 1916) for comparing the colours of bleached pulps which permits the results to be expressed numerically.

As standards for comparison plaster of Paris toned with other materials is used. The formulæ for the different standards are as follows:

Colour rating	Water	Plaster of Paris	Potassium chromate	Magnesia, powdered
100	120	107	0.0000	20
95	120	107	0.0245	20
90	120	214	0.1490	0
85	120	214	0.4660	0
80	120	214	0.6120	0
75	120	214	0.7770	0

The dry materials are first thoroughly mixed and then the water added; the well stirred paste is then poured into special brass wheels so designed that the plaster forms the exposed surface. When thoroughly dry, the plaster is turned down smooth with a chisel and finished with fine sand paper.

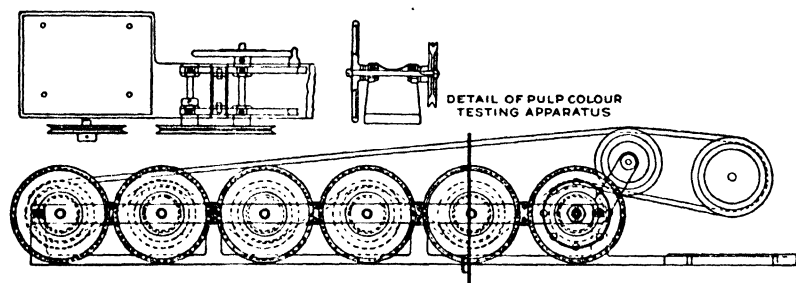


FIG. 101.—Colour testing apparatus for wood pulps.

These discs or plates are so constructed that they may be attached to a series of shafts and caused to rotate at 2500 r.p.m. A small disc of the pulp to be compared is fastened to the front of each standard plate, and while they are rotating the operator judges to which of the standards the pulp is nearest in shade. To give

satisfactory results, the apparatus should be placed in a dark room and the plates illuminated by a constant source of light, such as a daylight lamp.

The standard colours which would be satisfactory to one plant would not necessarily please another, but of the formulæ given that called 95 is satisfactory to the great majority of paper makers.

This apparatus, a general view of which is given in Fig. 101, is rather expensive, and if several grades of pulp are made or used, a different set of standard plates must be prepared for each grade. This is by no means a simple undertaking and the results are sometimes quite unsatisfactory. It is, however, in use in several plants in which it is reported to be giving good service.

**Bleaching Qualities.**—Two quite distinct methods for determining the bleach required by a sample of fibre are in general use. In one an excess of bleach solution is added, and when the fibre has assumed a satisfactory white colour, the excess is removed and the amount unconsumed determined by titration. This method has the defect that it is necessary to judge the colour of the fibre in the wet condition, and it is also true that it does not conform to actual operations in many bleaching plants.

The second method, which has been in satisfactory use for many years, is as follows: weigh out two 50 grm. samples of the fibre and also a small sample in which the moisture is to be determined. After the moisture has been ascertained calculate the air-dry fibre (10% moisture) in the 50 grm. samples and base the bleach consumption on this figure. Break up one of the 50 grm. samples in a little water by rubbing between the hands in a 6 × 8 in. battery jar, and when it is reduced to a uniform pulp dilute it to about 2 litres with water. From a solution of bleaching powder of known strength measure out a volume corresponding to the amount of bleach which it is estimated the fibre will require. Add this to the jar of fibre and keep the contents agitated and at a temperature of about 35° to 40° until the bleach is all used up. Throw the fibre on a 70 mesh wire screen and wash with a heavy stream of water until all knots or balls of fibre have been broken up, then make into sheets on a hand mould and dry on a steam heated drier, or if that is not available, on racks in the air. These sheets may then be compared with the standards for colour and if any very great difference

is found, a second bleaching should be made using the other 50 grm. sample and more or less bleach according to the indications of the first test.

This method of testing has been found to give results in very close agreement with actual operations in plants where practically this same method of bleaching is carried out. It is not so rapid as the first method, but is more reliable if properly conducted.

Since bleached fibre gradually changes in colour, the maintenance of standards for comparison with the bleached sheets presents quite a problem. Unless the colour comparing outfit, already described, is available the most practical method seems to be to cut a large number of samples from the same lot of unbleached fibre, determine very carefully the bleach required to bring it to the standard colour and then bleach one sample of this fibre each month and use it for a standard for the month following.

**Loss in Weight on Bleaching.**—In bleaching fibres there are two sources of loss, that caused by the mechanical handling of the fibre, which should be practically the same for all fibres, and that due to the oxidizing and solvent action of the bleach, which will vary according to the fibre and the severity of the bleaching treatment. A determination of this latter loss is, therefore, of considerable assistance in establishing the value of the fibre.

The determination may be carried out as follows:

Two samples of about 2 grm. are accurately weighed out and the moisture determined in one by drying to constant weight at 100° to 105°. The other is broken up to a pulp by moistening with a little water and rubbing between the thumb and fingers, and the pulp transferred to a small flask. Care and skill are required in this procedure, but after a little practice it can be done without loss of fibre. Bleach solution is next added to the flask from a burette, the amount added being that which the bleaching test has already shown will give the standard colour. The flask and its contents are kept at 35° to 40°, with occasional shaking, until the bleach is exhausted, and the fibre is then poured into a Gooch crucible, washed very completely with hot water, dried and weighed. The difference between the calculated bone dry weight of the unbleached fibre and that of the bleached sample represents the loss caused by the action of the bleach; it may for convenience be expressed as percentage of the unbleached weight.

**Sedimentation Test.**—The principle upon which this test depends is that the longer fibres are beaten, or the finer they are ground in the case of groundwood, the slower they part with the water with which they are mixed. The test is usually made by placing a known amount of fibre, reduced to a definite density with water, in some sort of a receptacle with a perforated bottom which retains the fibres but allows the water to pass through. A valve below the perforated bottom prevents the water from escaping until the desired time and permits of timing the outflow.

This test has not yet been thoroughly standardized and several different types of apparatus are in use, many of them being home-made. Fishburn and Weber (*Paper*, Oct. 11, 1916, 13) used a

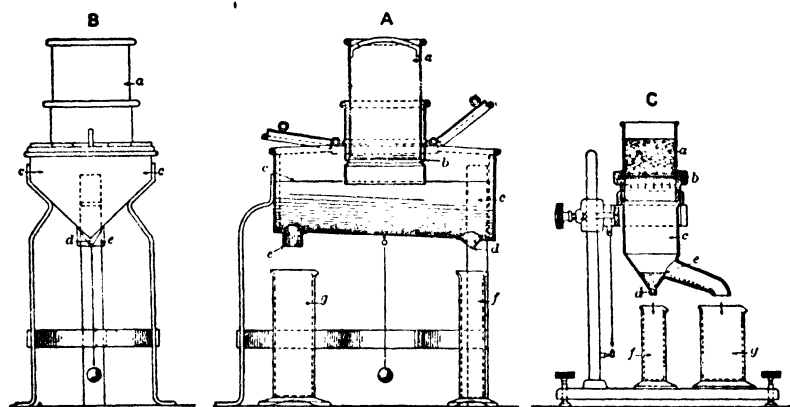


FIG. 102.—Riegler-Schopper sedimentation tester.

graduated glass cylinder for a receptacle and noted the time for the water level to drop 9½ in. In the Riegler-Schopper tester (U. S. Pat. 1,193,613, Aug. 8, 1916; *Paper*, Aug. 30, 1916) shown in Fig. 102, the water falls into a chamber with two outlets of different sizes and at different levels. The quantity of water discharged from the higher orifice, which is also larger than the other, is a measure of the degree of beating of the stock, or its natural slowness or quickness. The smaller orifice acts as a sort of cut off and makes the difference in the results more marked by taking care of the last slow drainings from the test sample.

**Dirt.**—If a sheet of pulp is wet and then examined by transmitted light the particles of dirt show very plainly. A convenient equip-

ment for this test consists of a box painted white inside and supplied with a glass top. Two or more electric light bulbs supply the light in the box. The samples of pulp should cover the entire top of the box, so that no glare from the lights bothers the eyes, and it is convenient to cut them of such a size that the dirt count may be expressed on the basis of some simple unit area. If desired the pulp can be weighed before moistening for the test and the count can then be based on weight.

There is a very large personal factor in this test and no two observers will quite agree as to what constitutes a dirt speck. For this reason it is well to have the work done by one man, for then the results will at least be comparable. It is also well to dig out the particles of dirt with a needle and note their nature, as for example, cinders, coal, bark, iron rust, etc., as this will often locate the chief cause of the trouble.



# ALIPHATIC ACIDS

REVISED BY HUGO SCHLATTER

The acids to be considered in this chapter comprise the lower members of the series of mono-basic saturated acids ( $C_nH_{2n}O_2$ ) and certain di-basic and hydroxy-acids. All these acids are found in nature as such or in the form of esters, or are formed through the fermentation of certain vegetable matters. The higher saturated and unsaturated fatty acids that are commercially important are treated in the chapter on fats, oils and waxes.

The mono-basic acids to be described are liquids at room temperature with the exception of trimethylacetic acid (m.p.  $35^\circ$ ). They are strongly corrosive and acid in character, even when largely diluted, as shown by their behaviour towards indicators and their neutralizing action upon carbonates and oxides; a few of them dissolve even such metals as iron and zinc. On the other hand, acetic, propionic and butyric acids, when entirely free from water, do not redden litmus paper. They are soluble in water; but solubility decreases with an increase in the number of carbon atoms in the molecule. The acids can be distilled without decomposition by themselves or with the aid of steam; some are appreciably volatile at ordinary temperatures. The salts are mostly colourless, unless formed with colour-producing metals, and are usually odourless; but the liability of some of them to hydrolyse rather easily often causes the apparently pure salt to have the odour of the acid from which it was produced. All the salts are decomposed by heating, generally leaving a residue of carbonate. The calcium and barium salts on heating give ketones containing one less carbon atoms than the number contained in two molecules of the acid, and the corresponding carbonate. When the salt-forming metal is easily reducible, the residue may be either an oxide or the metal.

The di-basic and hydroxy-acids are generally solid. Otherwise, their behaviour is similar to that of the mono-basic acids.

The following table shows the manner in which the neutral solutions of the potassium or sodium salts of the acids of this division are

affected by cold neutral solutions of barium, calcium and ferric chlorides, lead acetate and silver nitrate. The reactions refer to moderately concentrated solutions of the salts. When the precipitate is somewhat soluble in water, so as to render its production uncertain, the letter *P* is placed within parentheses. *S* signifies that the substance formed is soluble, and hence that no precipitate is obtained. Except when otherwise mentioned, the precipitates are

TABLE SHOWING THE REACTIONS OF THE SALTS OF SOME OF THE VEGETABLE ACIDS

Name of salt in solution	With barium chloride	With calcium chloride	With ferric chloride	With lead acetate	With silver nitrate	With hot Fehling's solution	With permanganate in cold acid solution	With hot concentrated sulphuric acid	Remarks
Acetate.	<i>S</i>	<i>S</i>	Red	<i>S</i>	( <i>P</i> )	<i>o</i>		Odour of acetic acid.	Silver salt not reduced on heating solution.
Formate.	<i>S</i>	<i>S</i>	Red	<i>S</i>	( <i>S</i> )		<i>R</i>	Carbon monoxide evolved.	Silver salt or solution reduced on heating.
Oxalate.	<i>P</i>	<i>P</i>	<i>S</i>	<i>P</i>	<i>P</i>	<i>o</i>	<i>R</i>	Carbon monoxide and dioxide evolved.	A yellow precipitate sometimes occurs on adding ferric chloride.
Lactate.	<i>S</i>	<i>S</i>	<i>S</i>	<i>S</i>	<i>S</i>	<i>o</i>	<i>R</i>	Carbon monoxide evolved.	See lactic acid.
Succinate.	( <i>P</i> )	( <i>S</i> )	Red-brown precipitate.	<i>P</i>	<i>P</i>	<i>o</i>	<i>o</i>	Brown colour. No change.	Barium and calcium salts precipitated on adding alcohol.
Malate.	<i>S</i>	( <i>S</i> )	<i>S</i>	<i>P</i>	<i>P</i>	<i>o</i>	<i>R</i>	Darkened.	Calcium salts insoluble in dilute alcohol.
Tartrate.	<i>P</i>	<i>P</i>	<i>S</i>	<i>P</i>	<i>P</i>	<i>o</i>	<i>R</i>	Charring.	Silver salt reduced on heating.
Citrate.	<i>P</i>	<i>P</i>	<i>S</i>	<i>P</i>	<i>P</i>	<i>o</i>	<i>o</i>	Carbon monoxide evolved.	Calcium salt precipitated on boiling and redissolved on cooling.
Aconitate.	( <i>P</i> )	( <i>P</i> )	..	<i>P</i>	<i>P</i>	..	..	Brown.	Calcium salt is soluble in two parts of water.
Meconate.	( <i>P</i> )	<i>P</i>	Red	<i>P</i>	<i>P</i>	..	..	..	Action of oxidising agents not recorded.

white. In addition to the reactions with the above metallic solutions, columns are added showing the reactions of the organic acids with other important reagents. *R* signifies "reduction" and *o* "no effect":

**Colour Reactions for Organic Acids.**—Some phenolic derivatives give colours with these acids. Messrs. H. J. H. Fenton and G. Barr have made a number of comparative experiments. They find that not only the acids, but their salts and esters can be detected, even in minute amount, by the reagents they used. The substance to be tested is mixed with strong sulphuric acid and the reagent. In testing dry material it is necessary to add a drop of water before deciding upon the result. The following is an abstract of the method (*Proc. Cambridge Philosoph. Soc.*, 1907, **14**, 386). In the table, *A* indicates the result on adding ammonium hydroxide after the reagents have been allowed to act for a short time. The changes are sometimes slow. Many acids were tried. The following, among others, gave no characteristic results: tartaric, citric, suberic, sebacic, mucic, malic, succinic, malonic, hippuric, acetic, butyric, stearic, amino-acetic, cinnamic.

Acid	Resorcinol	Phenol	Pyrogallol	1-2 Cresol
Formic.	Strong orange-red changing to blood-red. <i>A</i> , to dilute solution, green becoming purple.	Pink.	Pink, passing to orange-red and scarlet.	Light red.
Oxalic....	Slight yellow, passing to dark blue. <i>A</i> , change to pink.	Faint pink passing to red.	Dirty green passing to orange. <i>A</i> changes to intense blue, then purplish-brown.	Crimson. <i>A</i> , gives purple.
Lactic....	Yellow passing to orange. <i>A</i> gives fluorescent green.	Yellow or orange.	Orange.	Red-brown.

The following special methods with resorcinol are given by Mulliken (*Identif. Pure Org. Comps.*, Vol. I):

A small amount of the acid is mixed with a few drops of a freshly prepared solution of resorcinol, the dish placed on the water-bath and at intervals of half a minute portions of the liquid are removed and

the depth of colour noted. When the maximum is reached, the liquid is diluted cautiously with water and the colour changes noted.

**Citric acid**, pale greenish-blue, changing blue-green, then to pale impure green. Colour after dilution much paler.

**Tartaric acid**, pale blue-green for a moment, then pure intense green. Dilution with water gives orange-yellow.

**Malic acid**, momentary greenish-yellow changing to intense yellow, which is permanent. Dilution gives orange-yellow more intense than from the other two acids.

### Acetic Acid

This substance occurs in some plants and is a frequent product in chemical reactions. It is produced by the acetic fermentation of sugar and by the limited oxidation of alcohol. A large quantity is obtained by the distillation of wood. The crude material from this source is usually termed "pyroligneous acid."

Acetic acid is a colourless liquid, strongly acid and pungent. It crystallises in transparent plates, melting at  $16.7^{\circ}$ , and hence is often termed "glacial acetic acid." Acetic acid remains liquid if cooled in a closed vessel, even below  $0^{\circ}$ , but on opening or shaking the vessel or dropping in a fragment of the solid acid, the whole solidifies and the temperature rises to  $16.7^{\circ}$ . A small addition of water lowers the m.p. of acetic acid very considerably, so that an acid containing 13% of water melts below  $0^{\circ}$ , and one containing 38% of water (corresponding to  $\text{C}_2\text{H}_4\text{O}_2 + 2\text{H}_2\text{O}$ ) has a m.p. of  $-24^{\circ}$ . More water raises the m.p.

Acetic acid boils at  $119^{\circ}$  and distils unchanged. In distilling hydrated acid the last fractions are absolute or nearly so.

Addition of water to acetic acid causes evolution of heat, and contraction in volume until the mixture contains about 23% of water. Acid of this strength has a higher sp. gr. than the glacial acid, so that either concentration or dilution causes a diminution. The sp. gr. of moderately concentrated solutions of acetic acid cannot be used in ascertaining their strength, but is of service in examining the dilute solutions.

The table on page 643 taken from the United States Pharmacopœia (9th decennial revision) shows the sp. gr. of acetic acid of different strengths, all figures being at  $15^{\circ}/15^{\circ}$ . It will be seen by the table

that acid of 100% and acid of approximately 43% will coincide in gravity.

Absolute acetic acid is miscible in all proportions with water, alcohol and ether. It is powerfully corrosive, dissolves many essential oils, camphor and resins, phenols, gelatin, nitrocellulose and many metallic salts insoluble in water. The liquid acid is not inflammable, but the vapour burns with a blue flame. Moeller and Thomas give 44° as the flash point of acetic acid.

Acetic acid is stable. The most powerful oxidising agents attack it with difficulty. Chromic acid has no effect on it; a solution of chromic acid in acetic acid is employed for the oxidation of hydrocarbons. Nitric acid has no action; chlorine converts it into chloroacetic acid.

According to Bousfield and Lowry (*Trans. Chem. Soc.*, 1911, 99, 1432) acetic acid is best purified by distilling from potassium permanganate, using a still head to retain the acids of higher b.p., and then freezing to remove the water. The purified acid melts at 16.60° and has a sp. gr. 1.05148 at 18°/4° and 1.04922 at 20°/4°; its maximum conductivity when mixed with water is  $\kappa_{18} = 0.0016415$ . The boiling point of the pure acid is 117.88°  $\pm$  0.05° under 760 mm.<sup>1</sup>

Per cent.	Sp. gr.	Per cent.	Sp. gr.	Per cent.	Sp. gr.
1	1.0015	19	1.0279	37	1.0501
2	1.0030	20	1.0293	38	1.0511
3	1.0045	21	1.0307	39	1.0522
4	1.0060	22	1.0320	40	1.0532
5	1.0075	23	1.0333	45	1.0580
6	1.0090	24	1.0346	50	1.0624
7	1.0105	25	1.0359	55	1.0662
8	1.0120	26	1.0372	60	1.0694
9	1.0135	27	1.0385	65	1.0721
10	1.0150	28	1.0397	70	1.0742
11	1.0165	29	1.0409	75	1.0755
12	1.0179	30	1.0421	80	1.0757
13	1.0193	31	1.0433	85	1.0748
14	1.0208	32	1.0445	90	1.0722
15	1.0222	33	1.0456	95	1.0669
16	1.0236	34	1.0468	100	1.0562
17	1.0251	35	1.0479		
18	1.0265	36	1.0490		

<sup>1</sup> Ordinary acetic acid contains traces of glyoxylic acid and hence gives the Adamkiewicz reaction (Hopkins and Cole, *Proc. Roy. Soc.*, 1901, 68, 21); the acid purified in the manner described above is stable to bromine and does not give the Adamkiewicz indication. Deterioration of such acid does not occur even when the acid is exposed to light or atmospheric oxidation.

**Detection of Acetic Acid and Acetates.**—Most of the acetates are soluble in water. A few oxyacetates ("basic" acetates) are insoluble; silver and mercurous acetates are sparingly soluble. Hence, acetic acid cannot be estimated or readily detected by precipitation. Free acetic acid may generally be recognised by its odour and other physical properties, or it may be neutralised by sodium hydroxide and examined by the following tests:

**Metallic acetates** give the following reactions:

Subjected to dry distillation, acetone, is given off, having a highly characteristic odour.

Heated in the solid state in admixture with arsenous oxide ( $\text{As}_2\text{O}_3$ ), acetates give the very characteristic odour of cacodylic oxide which strongly resembles that of garlic. Only a very minute amount of materials should be used in this test, as the products are very poisonous.

Heated with sulphuric or phosphoric acid, acetic acid is evolved.

Heated with alcohol and concentrated sulphuric acid, the fragrant and characteristic ethyl acetate (acetic ether) is produced.

The neutral solution, on treatment with ferric nitrate or ferric chloride, avoiding excess, gives a deep-red liquid containing ferric acetate. This is decomposed on boiling, the liquid becoming colourless and depositing reddish-brown ferric oxyacetate. The reaction is imperfect if the iron solution is added in excess. The cold red liquid is not decolourised on addition of mercuric chloride (distinction between acetates and thiocyanates); and is not taken up by ether on agitation (distinction from thiocyanates); but the colour is readily destroyed on addition of cold dilute sulphuric or hydrochloric acid (distinction from meconates).

Insoluble (basic) acetates may be converted into sodium acetate by boiling with sodium carbonate and filtering off the insoluble carbonate.

Acetates containing nitrogenous bases respond, as a rule, to the foregoing tests, but the acetic esters do not. The latter can, however, be saponified by alcoholic alkali (see page 304), and after distilling off the alcohol the acetate can be examined.

**Analysis of Acetic Acid and Acetates.**—For samples consisting only of acetic acid and water the sp. gr. will often furnish sufficient information or the liquid may be titrated.

Phenolphthalein is applicable as indicator, sodium acetate being neutral to it, but alkaline to litmus. The end-reaction is sharp. Highly-coloured liquids, such as vinegar, may be largely diluted before titrating, as the delicacy of the reaction is but little diminished.

Methyl orange and phenacetolin are not suitable indicators for titrating acetic acid.

Acetates containing metals of the alkalis and alkaline earths are converted into carbonates on ignition. In many cases the amount of acetate originally present may be ascertained by titrating with standard acid, the residue of the ignition. Each cubic centimeter of normal acid required for neutralisation represents 0.060 grm. of acetic acid in the sample.

Salts of metals completely precipitated by sodium carbonate (*e. g.*, calcium, lead, iron) may be decomposed by a known quantity of it, the liquid well boiled, filtered, and the filtrate titrated with standard acid. The loss of alkalinity represents the acetic acid originally present as an acetate. Before adding the sodium carbonate the solution must be neutral.

In presence of salts of inorganic acids, the last method is valueless, but a modification may be employed: the excess of sodium carbonate is neutralised by hydrochloric acid, the liquid evaporated to dryness, the residue gently ignited, and the resultant carbonate titrated with standard acid. Each cubic centimeter of standard acid used represents 0.060 grm. of acetic acid. Other organic acids that may be present will be included as acetic acid.

Free acetic acid may also be estimated by adding excess of pure precipitated barium carbonate to the solution. The liquid is well boiled, filtered, and the barium in the filtrate precipitated by dilute sulphuric acid. 233 parts of precipitate obtained represent 120 of acetic acid in the sample taken. This process is applicable in presence of oxalic, phosphoric, sulphuric and other *free* acids forming insoluble barium salts, but is useless in presence of soluble oxalates, phosphates and sulphates. The method is available in presence of alkali chlorides, but not in presence of free hydrochloric acid, unless the solution is previously treated with excess of silver sulphate. Acetates and chlorides of metals of the alkalis and the alkaline earths do not interfere, but acetates and other salts of iron, aluminum and other metals precipitable by barium carbonate must be absent.

The estimation of acetic acid in acetates is best effected by distilling the salt to dryness with a moderate excess of sulphuric acid or with sodium hydrogen sulphate. Water should then be added to the contents of the retort and the distillation repeated. A third, and even a fourth distillation will sometimes be necessary, as the last traces of acetic acid are volatilised with difficulty.

In presence of chlorides, excess of silver sulphate should be added before commencing the distillation.

In presence of sugar or other substances liable to decomposition by sulphuric acid, phosphoric acid should be substituted. Care should be taken that the phosphoric acid used is free from nitric and other volatile acids. This is best insured by adding a little ammonia and heating the acid to fusion in a platinum crucible.

For the estimation of acetic acid in presence of its homologues, see the analysis of calcium acetate.

**Pyroligneous Acid.**—Pyroligneous acid or wood vinegar is the crude acetic acid obtained by the distillation of wood. It is a very complex product, containing, among other substances, homologues of acetic acid from formic to caproic acid; crotonic and angelic acids; furfural; substances of indefinite nature called "wood-oils;" pyrocatechol; acetone and other ketones of the acetic and oleic series; methyl alcohol and the other constituents of wood-spirit. By neutralising the crude product with lime and distilling, the volatile substances of indifferent nature are removed. When partially concentrated, the solution is faintly acidified with hydrochloric acid, when creosote and various tarry matters separate out; and the clear liquid on evaporation to dryness yields a brownish residue, which is heated to about  $230^{\circ}$  to decompose the empyreumatic products. On distillation with hydrochloric acid a comparatively pure acid may be obtained, which can be further purified by rectification with a little potassium dichromate. A better product is said to be obtainable by converting the acid into a sodium salt, heating to destroy tarry matters and distilling with hydrochloric or sulphuric acid.

The empyreumatic odour of acetic acid derived from the dry distillation of wood is in great measure due to furfural, vapours of which are always produced if a warm mixture of sulphuric acid and water is

poured on bran or sawdust, or if bran is distilled with an equal weight of sulphuric acid and 3 parts of water. If the vapours of furfural are evolved in a beaker covered with filter-paper soaked in aniline, the latter will turn red, but this soon disappears. This reaction may be employed for the detection of furfural which may be removed from pyroligneous acid by agitating the liquid with 3% by volume of benzene.

Pyroligneous acid differs much in strength according to the kind and state of division of the wood used for distillation, and is also affected by the construction of the retorts. Lopwood yields stronger acid and less tarry and resinous matters than spent dye-woods and sawdust, even though of the same kind.

Pyroligneous acid from finely divided wood has a sp. gr. of 1.040 to 1.045, and contains, on an average, about 4.5% of acetic acid. The product of the distillation of lop-timber contains an average of 7.75% of real acid.

The strength of pyroligneous acid may be ascertained by titration with standard alkali and phenolphthalein, but the liquid is frequently too dark in colour to permit of the end-reaction being readily observed. Calcium and sodium sulphates and acetates are frequently present. In the absence of sulphates, pyroligneous acid is best analyzed by treatment with excess of barium carbonate, with estimation of the dissolved barium as sulphate.

**Commercial acetic acid** ranges in strength from the nearly absolute glacial acid to the weakest vinegar. The proportion of real acetic acid may be ascertained by the methods already described: in certain cases by the sp. gr.; and in the case of glacial acid by the solidifying point.

The analysis of glacial acetic acid, pyroligneous acid and vinegar is described in the respective sections treating of these products.

Commercial acetic acid is often prepared by distilling sodium or calcium acetate with sulphuric or hydrochloric acid. It is liable to contain the following impurities:

*Sulphuric acid and sulphates*, indicated and estimated by addition of barium chloride, which in their presence throws down white barium sulphate.

*Sulphurous acid*, indicated by adding barium chloride in excess, filtering from any precipitate, and adding bromine water to the

clear filtrate. An additional precipitate of barium sulphate indicates the previous presence of sulphurous acid, and from its weight the amount of impurity can be calculated.

*Hydrochloric acid and chlorides*, detected and estimated by addition of silver nitrate.

*Copper and lead*, detected by evaporating a considerable bulk of the sample to a small volume, diluting with water, adding a few drops of hydrochloric acid, and passing in hydrogen sulphide which produces a black or brown colouration or precipitate in presence of lead or copper. If much organic matter is present, the evaporation should be carried to dryness and the residue ignited in porcelain. The heavy metals are then sought for in the residue in the manner described on page 65. A delicate test for copper is the red-brown precipitate or coloration produced by potassium ferrocyanide in the original liquid, or the same concentrated and then diluted with water. If iron is present in such quantity as to give a blue precipitate and thus interfere with the reaction, it must first be removed by addition of bromine water and excess of ammonia, and copper sought for in the filtrate after acidifying with acetic or hydrochloric acid. Samples of pickles suspected of being coloured with copper should be moistened with sulphuric acid, ignited, and the ash dissolved in nitric acid, and tested in acid solution with potassium ferrocyanide, after separation of the iron and phosphates with ammonia. The copper can be determined by electro-deposition on the inside of a platinum crucible by an electric current. *Tin* and *zinc* have been occasionally met with in acetic acid and vinegar.

Enz (*Apoth. Zeit.*, 1912, 27, 942) states that a supply of dilute acetic acid (sp. gr. 1.041) used in the preparation of pharmaceutical products contained 0.095% of alumina in the form of aluminium acetate.

*Salts of calcium* are detected by partially neutralising the solution with ammonia and adding ammonium oxalate, which will produce a white precipitate of calcium oxalate.

*Empyreumatic and indefinite organic substances* may be detected by exactly neutralising the acid with sodium carbonate and tasting and smelling the warmed liquid. The neutralised acid gives a precipitate when heated to boiling with ammonio-silver nitrate, and the original acid darkens when heated to boiling with an equal volume of concentrated sulphuric acid, if the above impurities are

present. A comparative estimate of the proportion of empyreumatic impurities present may be made by diluting 10 c.c. of the sample to 400 c.c. with water, adding hydrochloric acid, and titrating with permanganate till the pink colour is permanent for 1 minute.

*Formic acid* frequently occurs in acetic acid. The estimation of it has been investigated by H. Ost and F. Klein (*Chem. Zeit.*, 1908, **32**, 815), who compared several processes, such as neutralising with alkali and titrating with permanganate; oxidising with standard chromic acid and titrating for the excess of this acid; treating with mercuric chloride and weighing the separated metal. These methods are fairly accordant, and probably in absence of substances (other than formic acid) capable of reducing permanganate, the permanganate method is the best. (See also pages 687 and 688.) Ost and Klein found somewhat over 0.5% formic acid in some samples. This cannot be removed completely by distillation or by direct action of potassium permanganate on the acid, but is best removed by crystallization.

Pikos showed that it was quite easy commercially to produce acetic acid free from this impurity. In spite of this, Fincke (*Apoth. Zeit.*, 1910, 727) states that formic acid is still an impurity which is usually present (0.018 to 0.806%) in the acid used for pharmaceutical purposes. To estimate the formic acid present, Fincke operates as follows: 5 c.c. of the glacial acid, 5 grm. of sodium acetate, 40 c.c. of 1 in 20 solution of mercuric chloride and 30 c.c. of water are heated in an Erlenmeyer flask fitted with a reflux condenser for 2 hours in a water-bath, the part of the flask containing the liquid being fully immersed. The precipitated calomel is collected in a Gooch crucible, dried and weighed; its weight multiplied by 0.0977 gives the weight of formic acid. According to Bayer (*Pharm. Post*, **51**, 393) formic acid is detected in acetic acid by means of bromine water ( $\text{HCOOH} + \text{Br}_2 = \text{CO}_2 + 2\text{HBr}$ ). He adds bromine water to a solution of 2 to 3 grm. of sodium acetate in 10 c.c. of water until a yellow colour is produced and follows this with the addition of 0.5 c.c. of the acetic acid to be tested. If formic acid is present, the yellow colour disappears; if absent, the colour is intensified. The test is sensitive to 0.1% of formic acid. Bromine had previously been recommended by Orton, Edwards and King for estimating the freedom of acetic acid from impurities (*Trans. Chem. Soc.*, 1911, **99**, 1178). For this purpose a standard solution of

bromine (about N/5) is made in pure acetic acid; 5 c.c. of this solution are added to 45 c.c. of the acetic acid to be tested and 50 c.c. of water are introduced. The mixture is placed in the dark in a bath at 16° and the fall in titre, which is a measure of the impurities present, is estimated by determining the free bromine at intervals with hydriodic acid and N/50 thiosulphate.

*General fixed impurities* are detected and estimated by evaporating a known volume of the sample to dryness and weighing the residue.

For the *detection and estimation of acetic anhydride* when present in acetic acid in small quantities Edwards and Orton (*Trans. Chem. Soc.*, 1911, 99, 1181) make use of the fact that 2:4-dichloraniline combines rapidly with acetic anhydride but not with acetic acid at the ordinary temperature. The resulting anilide is readily and quantitatively converted into a chloramine, and the accurate estimation of the latter is extremely easy owing to its quantitatively liberating iodine from hydriodic acid, the iodine being determined as usual by means of thiosulphate.

The process is as follows: 2 grm. of 2:4-dichloraniline (or a quantity equivalent to about 3 times the amount of the acetic anhydride supposed to be present) is added to 100 c.c. of the acetic acid to be tested. The mixture is left overnight in a bath at 16°.

The mixture is then diluted with water so as to contain about 20% of acetic acid and the aniline (partly) and the anilide (wholly) extracted with chloroform. For each 100 c.c. of the diluted liquid, first 10 c.c., secondly 7 c.c. and lastly 5 c.c. of chloroform are used. The unchanged aniline is now withdrawn from the chloroform by shaking for a few minutes with 10% hydrochloric acid, 160–200 c.c. being used for each gram of the aniline originally present. The chloroform is carefully drawn off in a separating funnel and the hydrochloric acid washed twice with 1 c.c. of chloroform. The chloroform solution is now mixed with an equal volume of pure acetic acid, and dilute bleaching powder solution slowly added (3 to 5 times that required to convert the anilide to chloramine). The volume of the bleaching powder solution should be equal to the volume of the glacial acetic acid, that is, the acetic acid is diluted to 50%, a concentration at which the formation of chloramine is complete. A N/20 bleaching powder solution is now added in sufficient quantity

to dilute the aqueous acetic acid to 20% acetic acid. For example, if 30 c.c. of glacial acetic acid were added to the chloroform solution of the anilide, then 30 c.c. of bleaching powder solution would be required for the first addition and 90 c.c. of N/20 bleaching powder for the second. Chloroform withdraws the chloroamine completely from 20% acetic acid. Any marked development of red colour at this stage shows that the aniline has not been properly separated.

The chloroform is drawn off into 30 to 40 c.c. of N/20 bleaching powder solution, with which it is shaken to free it from acetic acid. Finally the chloroform is evaporated in a current of pure dust-free air at a temperature not exceeding 25°. The residue is dissolved in chloroform (5–10 c.c.) and treated with 5% potassium iodide and a little acetic acid; the iodine liberated is titrated with N/20 thio-sulphate.

**Glacial acetic acid** (absolute acetic acid). The properties of this substance have been already described.

Commercial glacial acetic acid should contain at least 97% of the absolute acid. This may be ascertained by agitating 1 volume of the sample with 9 of spirits of turpentine. Complete solution occurs if the strength is 97% or above. Samples containing 99.5% of absolute acid are miscible with spirits of turpentine in all proportions. Oil of lemon, if freshly distilled, may be employed instead of turpentine.

A more delicate test for water is to treat the sample in a dry test-tube with an equal volume of carbon disulphide, and to warm the mixture in the hand for a few minutes. The liquid will be turbid if any water is present in the sample.

N. Schoorl (*Pharm. Weekblad*, 54, 945) considers tests with carbon disulphide as unreliable on account of its too low solubility and too high volatility. He recommends instead carbon tetrachloride, which is miscible in all proportions with anhydrous acetic acid, but not with acetic acid containing water. A little iodine is added to show separation into two layers (when water is present) more distinctly by colour difference, the aqueous layer being yellow-brown.

The influence of water on the m.p. of glacial acetic acid is shown in the following table by Rudorff (*Pharm. Jour.* [3], 1872, 2, 241):

Solidifying point. °C.	Water to 100 parts of real $C_2H_4O_2$	Solidifying point. °C.	Water to 100 parts of real $C_2H_4O_2$
+16.70	0.0	6.25	8.0
16.65	0.5	5.30	9.0
14.80	1.0	4.30	10.0
14.00	1.5	3.60	11.0
13.25	2.0	2.70	12.0
11.95	3.0	-0.20	15.0
10.50	4.0	-2.60	18.0
9.40	5.0	-5.10	21.0
8.20	6.0	-7.40	24.0
7.10	7.0		

The strength of glacial acetic acid may also be ascertained as on page 644. The sp. gr. is not an indication of value. Impurities may be sought for as on page 647.

According to the British Pharmacopœia (1914) the following are the requirements for acetic acid (glacial). It crystallises when sufficiently cooled and does not entirely remelt until the temperature rises above  $14.7^{\circ}$ . 1 gram. diluted with 50 c.c. of water requires for neutralisation not less than 32.9 c.c. of N/2 solution of sodium hydroxide. It leaves no residue on evaporation and yields no characteristic reaction for lead, copper, arsenic, chlorides, nitrates, sulphates or sulphites. It does not immediately darken in colour when neutralised with *solution of ammonia* and warmed with *solution of silver nitrate* (absence of formates). 2 c.c. of glacial acetic acid do not completely decolorise a mixture of 3 drops of *solution of potassium permanganate* and 10 c.c. of water within half a minute (limit of empyreumatic matter).

According to a method published by the Association of Official Agricultural Chemists (September, 1920), *acetic acid* (total acidity) in *tanning liquors* is determined as follows:

**Reagents.** (a) *Hematin Solution*.—Digest 0.5 gram. of hematin in 100 c.c. of cold neutral 95% alcohol.

(b) *Gelatin Solution*.—Dissolve 10 gram. of gelatin in hot water, cool, add 25 c.c. of 95% alcohol and dilute. If the gelatin solution is acid or alkaline, neutralise with N/10 sodium hydroxide or acetic acid, respectively, using hematin solution as an indicator, and make up to 1 litre.

(c) *Kaolin*.—Digest with dilute hydrochloric acid, wash until it is neutral to phenolphthalein and does not yield more than 1 mg. of

soluble solids per 100 c.c. of filtrate of a 1% suspension after an hour's digestion at 20° C.

**Estimation.**—Add 25 c.c. of the gelatin solution to 25 c.c. of the tanning liquor in a stoppered cylinder, dilute with water to 250 c.c., add 15 grm. of the kaolin and shake vigorously. Allow the mixture to settle for at least 15 minutes, remove 30 c.c. of the supernatant liquid, dilute with 50 c.c. of water and titrate with N/10 sodium hydroxide, using the hematin solution as an indicator. Each cubic centimeter of N/10 sodium hydroxide is equivalent to 0.2% of acid, calculated as acetic, in the liquor.

## VINEGAR

Properly speaking, vinegar is a more or less coloured liquid, consisting essentially of dilute acetic acid, obtained by the oxidation of alcohol. Sometimes the term is improperly extended to pyroligneous acid, or "wood vinegar," whilst acetic acid is called "distilled vinegar." In the United States, vinegar made by oxidising dilute alcohol is often called "spirit" vinegar, and as the dilute alcohol is sometimes called "low wines" the vinegar is called "wine" vinegar, but such a misleading name is now generally forbidden by laws against misbranding.

The reaction between alcohol and oxygen takes place under the influence of platinum-black and some other substances, but the formation of vinegar from alcoholic liquids usually depends on microorganisms. Mechanical arrangements are employed to expose a large surface of the alcoholic liquid to the air, so as to diminish the time required for acetification.

Besides acetic acid, vinegar often normally contains more or less of other organic acids, sugar, dextrin and colouring matters. The agreeable aromatic smell is doubtless due to esters, and is sometimes imitated by direct addition of ethyl acetate.

The sp. gr. of vinegar is of no value as an indication of its strength in acetic acid, as the proportion of extractive matter differs much in vinegar from various sources. The obsolete "proof vinegar" of the (British) excise contains the equivalent of about 5% of acetic anhydride, or 6% of the absolute acid, and has a sp. gr. of 1.019.

British manufacturers distinguish their products by "trade-numbers," viz., 24, 20, 18 and 16. These numbers originally indi-

cated the price of the vinegar in pence per gallon, but were subsequently said to have indicated the number of grains of pure dry sodium carbonate required for the neutralisation of 1 fluid ounce. The tradition and usage of the vinegar industry is against this interpretation. .

Genuine vinegar of good quality will not contain much less than 5% of absolute acetic acid. Though the percentage depends on the origin of the vinegar. Cider vinegar being naturally the weakest and wine vinegar the strongest in acetic acid.

Russell and Hodgson (*Analyst*, 1910, 35, 346) give the following analyses of 12 samples of genuine malt vinegar and 2 samples of wood vinegar:

	Malt vinegars		Wood vinegars	
	Lowest	Highest	1	2
Sp. gr. ....	1013.7	1022.1	1012.1	1012.4
Acetic acid, % .....	3.85	6.36	2.20	2.30
Total solids, % .....	1.47	3.15	0.47	0.43
Ash, % .....	0.18	0.60	0.04	0.03
Alkalinity of ash (as $K_2O$ , %).	0.016	0.040	0.014	0.022
$P_2O_5$ , % .....	0.047	0.092	<i>Nil</i>	<i>Nil</i>

In estimating total solids it was found that the residues retained from 0.15 to 0.22% of acetic acid and that a neutral residue was obtained only when the latter was moistened with water and evaporated 3 times in succession. It has been suggested that 4% of acetic acid and 0.05% of phosphoric acid should be taken as the minimum standards for malt vinegar.

The proportion of acetic acid in vinegar may be ascertained by titration with standard alkali hydroxide, phenolphthalein being used as an indicator. Other methods are described on page 644.

**Volatile Acids in Wines and Vinegars.**—Gore (*U. S. Bureau of Chem.*, 1909, Circular No. 44) describes the apparatus shown in Fig. 103 for the estimation of volatile acids in wines and vinegars. The apparatus is a modification of that due to Hortvet-Sellier, in which a copper flask is substituted for the outer glass flask and a constant-feed device for the flask has been added. A small ridge is also blown in the inner flask to form a shoulder for a rubber gasket and the dropping funnel has been eliminated as unnecessary. The constant

water feed is operated by running the supply water through tube *a*, which passes through tube *b*. The overflow passes through *b* and rises through *c* to the small basin *d* which is connected to the drain. Distilled water should be used which has been largely freed from

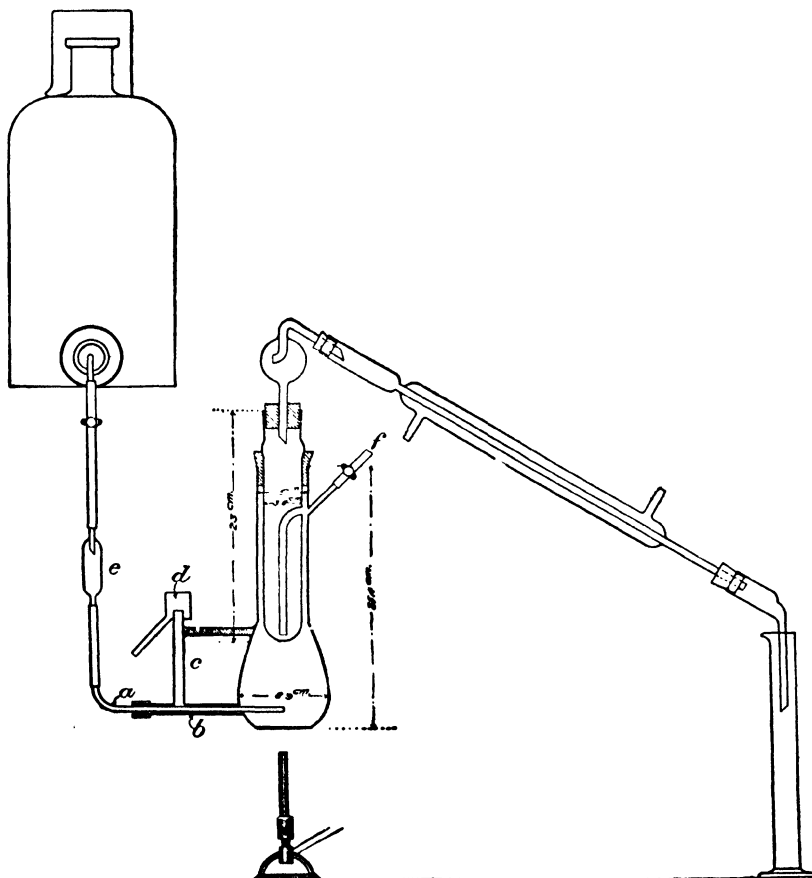


FIG. 103.—Modified Hortvet-Sellier apparatus.

carbon dioxide by passing a rapid stream of air through it for 20 to 30 minutes. The rate of flow of the water is regulated by comparing the rate of drip in the small sight-tube *e* with that from the condenser. Experiments with the apparatus showed that when tap-

water was supplied to the generator there was an error, due to the presence of carbon dioxide in the distillate equal to about 0.3 c.c. of N/10 alkali in 100 c.c., when phenolphthalein was used as indicator. When ordinary distilled water was used the error was 0.15 c.c., and when the carbon dioxide was removed by aeration, as described, the error was only 0.05 c.c. This error is constant and may be subtracted from the results. The operation is carried out as follows: 10 c.c. of the sample are placed in the inner flask which should be removed from the outer flask and be quite cool. If the sample is noticeably charged with carbon dioxide, this should be removed by passing a current of air through it, by attaching to the flask a stopper fitted with a glass tube connected with suction. The air passes in through the side tube of the flask and washes out the carbon dioxide without removing appreciable quantities of volatile acids. The flask is then connected with the distilling bulb, and placed in the outer flask, tube *f* of the latter being open, all connections are made tight and the tube *f* is then closed. In the case of wines about 100 c.c. of the distillate are collected, in the case of vinegars 200 to 300 c.c. The distillate is titrated with standard alkali, free from carbonate, using phenolphthalein as indicator, and 0.05 c.c. is subtracted from the number of c.c. of N/10 alkali used for each 100 c.c. of distillate. About 15 minutes are required for the estimation of volatile acids in wine, and from 30 to 45 minutes in the case of vinegar. The volume of liquid in the inner flask increases but slightly during the determination.

**Mineral Acids.**—According to Repiton (*Monit. Scient.*, 1909, 23, 172) a portion of the sample is titrated with a solution of calcium saccharate, using phenolphthalein as indicator, to ascertain the total acidity. Another portion is neutralised with sodium hydroxide, free from carbonate, then acidified with phosphoric acid and subjected to distillation until the distillate is no longer acid. The distillate is then titrated with calcium saccharate; if the result obtained is identical with the total acidity, free mineral acids are probably not present. If there be a difference, sulphuric acid and hydrochloric acid should be estimated in the ordinary way. Nitric acid is not likely to be present. Phosphoric acid is estimated by treating a portion of the vinegar with calcium carbonate, evaporating to dryness, incinerating and precipitating the nitric acid solution of the ash in the usual manner.

**Wine vinegar** differs in colour according as its origin is white or red

wine, that derived from the former being most esteemed. It contains from 6% to 12% of absolute acetic acid, has a relatively low sp. gr. (1.014 to 1.022), and an extract ranging from 1.7% to 2.4% (average 2.05). If the "extract" or residue left on evaporation is treated with alcohol, nearly everything dissolves except a granular residue of crude tartar, whilst vinegars made from malt or sugar leave a more or less glutinous residue, only sparingly soluble in alcohol. The amount of "tartar" (potassium hydrogen tartrate) contained in wine vinegar averages 0.25%. Its presence is peculiar to wine vinegar. The tartar may be proved to be such by pouring off the alcohol and dissolving the residue in a small quantity of hot water. On cooling the aqueous solution and stirring the sides of the vessel with a glass rod, potassium hydrogen tartrate will be deposited in streaks in the track of the rod. An addition of an equal volume of alcohol makes the reaction more delicate. Tartaric acid is occasionally added to vinegar as an adulterant, in which case the residue left on evaporation at a steam heat is viscous and highly acid. By treatment with proof-spirit any free tartaric acid is dissolved, and may be detected in the solution by adding a solution of potassium acetate in proof-spirit and stirring with a glass rod. In presence of tartaric acid, streaks and probably a distinct precipitate of potassium hydrogen tartrate will be produced. By titrating the precipitate with standard alkali, the amount of free tartaric acid in the vinegar can be estimated.

Fleury (*J. Pharm. Chim.*, 1910, 2, 264) states that as inositol is not decomposed during the acetous fermentation, its presence can be used as a test for the genuineness of wine vinegars, which always give a decided reaction of inositol. 100 c.c. of the vinegar are evaporated nearly to dryness, the residue is taken up with 50 c.c. of water, neutralised with sodium hydroxide and triturated with 3 gm. of barium hydroxide. The liquid is separated and the precipitate washed with 20 to 30 c.c. of baryta water. The liquid and washings are freed from barium by means of carbon dioxide or sulphuric acid and the defecation is completed by adding 10 c.c. of normal lead acetate solution (1:3). The clear liquid is made up to 100 c.c. and 10 c.c. of official basic lead acetate and a solution of 2 gm. of neutral cadmium nitrate added. The precipitate, which contains the inositol, is decomposed with hydrogen sulphide and the filtrate evaporated to a syrup. The residue is taken up with 20 c.c. of absolute alcohol and 5 c.c. of

dry ether added. After 1 or 2 days' standing, the solution is tested for inositol by the usual methods.

**Cider vinegar** is yellowish, has an odour of apples, a sp. gr. of 1.013 to 1.015, and contains 3.5% to 6% of acetic acid. On evaporation to dryness it yields from 1.5% to 1.8% of a mucilaginous extract, smelling and tasting of baked apples, and containing malic but no tartaric acid. Cider vinegar usually gives slight precipitates with barium chloride, silver nitrate and ammonium oxalate, and always with lead acetate. Perry vinegar presents similar characteristics.

The frequent imitation of cider vinegar by a mixture of acetic acid and water with addition of colouring matter (generally caramel) has led to much investigation as to the means of detecting the fraud. Among the more important contributions to this subject are papers by Allen and Moor (*Analyst*, 1893, 18, 240), C. S. Cox (*Analyst*, 1894, 19, 89), and A. W. Smith (*J. Amer. Chem. Soc.*, 1898, 20, 3). Cox gives the analytic results on 20 samples of cider vinegar and 4 samples of unfermented cider. The acidity of the vinegar ranged from 2.28% to 8.4%, the solids from 1.34% to 4.0%, the ash from 0.25% to 0.52%. By recalculating these results by *Hehner's rule* it is found that the proportion of original solids of the juice ranged from 5.51% to 16.00% and the ash from 1.94% to 4.88%.

The distinction between unadulterated cider vinegar and the imitation made by adding colouring matter to dilute acetic acid can be easily made. The latter preparation leaves but little solid residue, almost no ash, and has but little flavour.

A. W. Smith finds that the ash of cider vinegar differs from that of most other vinegars in the following important points:

It commences to melt and volatilise at a comparatively low temperature and gives to a flame the potassium tint unobscured by that of sodium. It is low in chlorides and sulphates and high in carbonates and phosphates; about two-third of the phosphates are soluble in water. In the ash of other vinegars a much lower proportion of phosphates is soluble in water. The dilution of vinegar with natural water will be apt to reduce the soluble matter by the formation of calcium and magnesium phosphate.

From a study of fine cider vinegars F. E. Mott (*J. Ind. Eng. Chem.*, 1911, 3, 747) concludes that there is a relation between the contents of *lævulose* and of *dextrose* therein. If the amounts of *lævulose* and *dextrose* (percentage of total solids) be plotted on squared

paper as abscissæ and ordinates respectively, then a line drawn from the zero point through the point corresponding to 15% dextrose and 25% of lævulose will represent the maximum values for dextrose in relation to the corresponding values for lævulose in fine cider vinegar, and a higher value for dextrose indicates adulteration with a foreign dextrorotatory substance.

In estimating the lævulose and dextrose the following method is recommended: the total solids from 10 c.c. of the vinegar are dissolved in 25 c.c. of water, the solution made up to 100 c.c. and the reducing sugars estimated by Munson and Walker's method (see page 401). 50 c.c. of the vinegar are then treated with 5 c.c. of basic lead acetate solution and two teaspoonsful of animal charcoal, filtered and the filtrate polarised in a 200 mm. tube at 20°. From the polarisation ( $p$ ) expressed in Ventzke degrees, and the percentage of reducing sugars ( $R$ ) in the total solids, expressed as dextrose, the percentage of lævulose ( $L$ ) and of dextrose ( $D$ ) are calculated by means of the formulæ:

$$P = \frac{p \times 26.048}{\text{total solids}}; L = \frac{0.793 R - P}{2.08}; D = R - 0.915 L$$

Mott claims that pure cider vinegar contains about 0.6 as much dextrose as lævulose; consequently, if a suspected sample shows a dextrorotation of polarised light at 87°, it may be adulterated with dextrorotatory substances, usually glucose. On the other hand, a lævorotation at 87° is not conclusive evidence of purity. Mott estimates the reducing sugars by the polarimeter and by Munson and Walker's method after evaporation to remove aldehydes.

**Adulteration of Cider Vinegar with Distilled Vinegar.**—Pure cider vinegars contain volatile constituents which have a reducing action on Fehling's solution. These substances are not present in distilled vinegar or pyroligneous acid. A method based on this fact is given by S. L. Crawford (*J. Ind. Eng. Chem.*, 1913, 5, 845) as follows: 50 c.c. of the sample are diluted to 250 c.c., placed in a distilling flask, and 200 c.c. are distilled into a 250 c.c. flask. The distillate is then neutralised, made up to 250 c.c. and 50 c.c. are taken for estimation of the reducing substances by Munson and Walker's method. Calculated to the basis of a vinegar containing 4% acid, the distillate should contain from 0.11% to 0.15% of reducing substances calculated as invert sugar. Pure cider vinegars usually contain 0.007 grm. of formic acid

per 100 c.c. Pyroligneous acid would raise this figure, whilst distilled vinegar would lower it.

**Lead Number.**—The qualitative detection of adulteration of cider vinegar by measurement of the precipitate formed by lead acetate is regarded by many to be one of the most useful of tests. For this purpose a rapid process is given by Hortvet (*J. Amer. Chem. Soc.*, 1904, 26, 1532) as follows:

To 25 c.c. of the vinegar are added 2.5 c.c. of U.S.P. lead subacetate solution. The mixture is shaken and whirled in a graduated Hortvet tube in a centrifugal machine. The volume of the precipitate in the bottom of the tube is then read off and this volume in cubic centimeters is termed the *Hortvet lead number*. The lead numbers of pure cider vinegars have been found to range from 0.5 to 1.4 while adulterated cider vinegars give numbers from almost zero to 0.5, rarely more than 0.5.

The Hortvet tube is 15.3 cm. in length, and consists of a wide cylindrical portion 3 cm. in diameter, narrowed at the top to a neck which is 2 cm. in diameter, and at the bottom to a stem graduated in tenths to 5 c.c. Hortvet's centrifugal machine had a radius of 18.5 cm. and was run at a speed of 1600 r.p.m.

Winton's method (*J. Amer. Chem. Soc.*, 1906, 28, 1204) of estimating the lead number of vinegars has the advantage of not requiring the use of a centrifugal machine. This method gives most satisfactory results, but requires much more time than the Hortvet process, owing to the fact that the lead is estimated by a gravimetric method. Cider vinegars give Winton numbers of 0.075 to 0.290; malt vinegars much higher values, whilst distilled vinegars give much lower figures.

**Beer and malt vinegars** have a fairly high sp. gr. (1.014 to 1.022) and yield about 3 to 6% of extract, containing a notable proportion of phosphates. The acetic acid usually varies from 4 to 6%. Barium chloride and silver nitrate frequently give considerable precipitates, owing to the presence of sulphates and chlorides in the water used in the manufacture or from the sulphuric acid used for hydrolysing the grain. It is stated that some manufacturers colour spirit vinegar (see above) by soaking dark malt in it and designate the product as "malt vinegar."

**Glucose or sugar vinegar** is now extensively prepared from amylaceous materials by conversion with dilute acid, followed by

fermentation and acetification. Glucose vinegar usually contains dextrose, dextrin and, very often, calcium sulphate. Hence it reduces Fehling's solution and usually gives abundant precipitates with barium chloride and ammonium oxalate, and frequently with silver nitrate also. When mixed with 3 or 4 times its volume of strong alcohol, glucose vinegar gives a precipitate of *dextrin*. It is best to concentrate the sample before applying this test. Dextrose is best detected and estimated by evaporating 50 c.c. of the sample to a syrup and adding alcohol. The liquid is filtered, decolorised by boiling with animal charcoal, again filtered, the alcohol boiled off and the dextrose estimated by Fehling's solution.

Vinegar may be made by diluting acetic acid to suitable strength, colouring with burnt sugar, and flavouring with a little acetic ether. Such a product differs from malt vinegar in containing no phosphates, and from wine and cider vinegars in the absence of tartaric acid and malic acid, respectively.

Hehner regards the presence of aldehyde and alcohol, causing an abundant iodoform reaction in the distillate from the neutralised sample, as evidence of fermentation, and that the sample is true vinegar. Vinegar made from sugar contains hardly any proteins, whilst that from malt contains about 0.7%. Vinegar prepared by acid inversion of starches usually contains a high ash with sulphates. The ash of cane-sugar vinegar is readily fusible; that of a malt or a glucose vinegar does not readily fuse. Sugar vinegar yields an ash composed mainly of potassium salts, as raw cane-sugar is employed, not refined sugar. The estimation of potassium with a view to prove the presence of grain vinegar is useless, since both grain and raw sugar contain much potassium.

Alcohol always exists in a well-made fermentation vinegar, for manufacturers stop the process before the acetification is complete. Vinegar may diminish in strength on exposure to the air, and in extreme cases the whole of the acid may disappear in the course of a few weeks. This is due to the action of living acetic bacteria left in the vinegar. If the alcohol is all destroyed the change is likely to be much more rapid. Vinegar should contain alcohol not only for keeping purposes, but to insure a gradual formation of acetic ether, just the same as in wine after keeping. Fermentation vinegar might be distinguished in that way, but it is easy to add alcohol to imitate a fermentation vinegar. Some manufacturers add acetic ether.

There is a considerable amount of solid extract in fermentation vinegar, but in a mixture containing pyroligneous acid the quantity is often much less. The solid matter differs much according to the perfection of the fermentation, and affords an indication of some value, though not so great as the amount of ash, which does not change to a great extent through the fermentation. The proportion of sulphates will afford some information as to the probable use of glucose. The estimation of total nitrogen is a valuable criterion. Grain vinegars contain a notable amount of nitrogen, although some manufacturers attempt to remove nitrogenous matters. In estimating the total nitrogen by the Kjeldahl method, the vinegar is evaporated to dryness, or at any rate to a syrup, before adding the sulphuric acid. 25 c.c. of vinegar is a convenient quantity to employ. The nitrogen found can then be calculated to its equivalent of proteins by the usual factor; but probably much of the organic nitrogen of vinegar exists in other forms. In one case Allen found 10% of the nitrogen as ammonium salts. The proportions of all constituents will differ with the strength of the vinegar. A wort which originally contained 12% of sugar and other solids will contain more nitrogen, ash and phosphates than a vinegar which originally contained only 7% of sugar. Therefore, it is desirable to adopt Hehner's plan of calculating the various constituents upon the original solids of the vinegar; 60 parts of acetic acid are theoretically produced from 90 of glucose, and hence, if the acetic acid found be multiplied by 1.5, we obtain the amount of sugar from which that acetic acid was derived. Adding to the figure thus obtained the total extractive matters still contained in the vinegar, we obtain a number representing the "original solids" of the wort. Thus, if a vinegar contains 5.2% of acetic acid and 2.8% of extract, the original solids will be  $7.8 + 2.8 = 10.6$ . If the vinegar itself contained 0.08% of nitrogen, the original solids contained:

$$\frac{0.08 \times 100}{10.6} = 0.75\%$$

In this manner one can eliminate the differences caused by irregularity in the strength of various samples of vinegar and reduce the results to a kind of common denominator. As a matter of fact, the loss of acetic acid in the process of manufacture averages some 30%, so that the proportion of original solids calculated in the above man-

## SYNOPSIS OF RESULTS OF EXAMINATION OF TYPICAL SAMPLES OF VINEGAR

Figures are grams per 100 c.c.

Sample mark	A	B	C	D	E	F	G	H	I	J	K	L	M
Specific gravity.....	1.0203	1.0170	1.0228	1.0160	.....	1.0130	1.0185	1.0100	1.0160	1.0104	.....	1.0070	1.0104
Acetic acid.....	6.61	6.39	5.26	4.86	4.23	5.22	5.82	5.58	5.70	3.51	4.92	4.70	7.00
Total solids.....	2.81	2.67	3.96	2.31	2.70	1.56	2.45	2.98	2.09	1.52	1.76	0.21	0.10
Ash.....	0.55	0.34	0.40	0.47	0.34	0.30	0.39	0.30	0.43	0.27	0.278	0.04	0.015
Containing:													
Alkalinity (K <sub>2</sub> O).....	0.102	0.091	0.118	.....	0.024	0.03	.....	0.013	.....	0.080	.....	trace	trace
Phosphoric acid.....	0.066	0.077	0.093	0.057	0.105	0.064	0.041	0.017	0.024	0.010	0.016	0.009	none
Nitrogen.....	0.120	0.099	0.095	0.030	.....	0.052	0.097	0.104	0.062	0.014	0.016	.....	0.002
Proteins.....	0.756	0.624	0.598	0.024	.....	0.328	0.611	0.655	0.390	0.088	0.103	.....	0.013
"Original solids".....	12.73	12.26	11.85	9.60	9.35	9.39	11.18	11.35	10.64	6.81	10.02	7.26	10.60
Per 100 parts of original solids:													
Ash.....	4.32	2.78	3.37	4.02	3.64	3.20	3.49	2.64	4.04	3.94	2.77	0.55	0.14
Phosphoric acid.....	0.52	0.63	0.79	0.60	1.16	0.68	0.37	0.15	0.225	0.14	0.16	0.120	none
Nitrogen.....	0.95	0.816	0.80	1.03	.....	0.56	0.87	0.93	0.582	0.206	0.16	.....	0.019
= Proteins.....	5.98	5.14	5.04	6.40	.....	3.53	5.48	5.86	3.670	1.30	1.03	.....	0.120

B, C, D, and probably A appear to be from mixtures of malted and unmalted grain, the starch entirely hydrolysed by diastase. E is the average of the first seven samples reported by Hefner.

F and G are from mixtures of malted and unmalted grain with addition of sugar.

H and I are chiefly from rice hydrolysed by sulphuric acid.

J and K were made from sugar; J contained possibly a little malt.

L was reported by Dr. Hill as containing between 70 and 80% of wood acid.

M was a very pale vinegar made by mixing distilled vinegar with a little of the same sample undistilled. It possessed an appetising taste and smell.

ner is always below the truth. Hence a nearer approximation to accuracy would be obtained by multiplying the acetic acid by 2.25, instead of 1.5, before adding the extract, but the change would involve confusion, and it is best to adhere to the mode of calculation originally suggested by Hehner.

**Wood vinegar** is a name sometimes applied to pyroligneous acid, but more usually to a purer acetic acid coloured with caramel. Samples of crude pyroligneous acid containing respectively 8.4 and 8.7% of acid calculated as acetic acid were found by G. Frerichs (*Apoth. Zeit.*, 1913, 28, 525) to give distillates containing only 3.9 and 4.2% of acetic acid. From a comparison with older samples it is concluded that crude pyroligneous acid as now sold contains acids other than acetic acid and is of inferior quality to that obtained formerly or may consist of a waste product from which much of the acetic acid has already been distilled.

**Aromatic vinegar** was originally a product obtained by distilling a metallic acetate, usually crystallised cupric acetate. The presence of acetone and other substances imparted an agreeable aroma. A small addition of camphor or essential oil was often made. The name is now applied to a solution of various essential oils in concentrated acetic acid.

*Mineral Acids in Vinegar.*—Very weak vinegar is liable to a putrid fermentation, to prevent which the addition of 1 gallon of sulphuric acid to 1000 gallons of vinegar (about 0.185%) was permitted by a British Excise regulation of 1818, although this proportion was frequently exceeded. This addition is now known to be unnecessary with good vinegar and is probably obsolete. In addition to sulphuric acid, hydrochloric acid has been occasionally added to vinegar, but the adulteration of vinegar with mineral acids is now very rarely practised<sup>1</sup> and where detected becomes the subject of prosecutions under the British Food and Drugs Acts.

For detecting mineral acids in vinegar several tests have been devised, but the most are either untrustworthy or deficient in delicacy. Some are applicable to the detection of sulphuric acid only, whilst others include hydrochloric and other mineral acids also. The employment of barium chloride and silver nitrate for the detection of sulphuric and hydrochloric acids, respectively, has led several analysts into error, owing to the normal presence of sulphates

<sup>1</sup> Any use of mineral acids in vinegar is not permissible in the U. S.—*American Editor*.

and chlorides in the water employed in the manufacture of the vinegar.

Another circumstance which complicates the problem is that the addition of a mineral acid in moderate quantity merely decomposes the acetates naturally present in the vinegar, with liberation of acetic acid and formation of sulphates or chlorides. Hence, only the acid beyond that required for the decomposition of the acetates can exist in the free state, and to the presence of such free mineral acids only can objection reasonably be taken, unless the mineral acid used is contaminated with arsenic.

Acetates and most other salts of organic acids decompose by ignition into carbonates, having an alkaline reaction to litmus, whilst sulphates and chlorides of the lighter metals are unchanged on ignition and possess a neutral reaction. Hence, if the ash of a vinegar has a sensibly alkaline reaction, acetates must have been present in the original vinegar and no free sulphuric or hydrochloric acid. To determine the amount of free mineral acid it is sufficient to neutralise the vinegar with standard sodium hydroxide before evaporation to dryness (the same process serves for a determination of the total free acid), ignite the residue, and titrate the aqueous solution of the ash with standard acid. If the free acid originally present was wholly organic, the ash will contain an equivalent amount of alkaline carbonate, which will require an amount of standard acid for its neutralisation exactly equivalent to the amount of standard alkali originally added to the vinegar. Any deficiency in the amount of standard acid required for neutralisation is due to the *free mineral acid* originally present in the vinegar. More accurate results are obtained if the amount of standard alkali added before evaporation is insufficient for the complete saturation of the acetic acid, but more than enough for the neutralisation of all mineral and fixed organic acids which may be present. By thus proceeding, N/10 alkali and acid may be employed (50 c.c. of the vinegar being used), and thus sharper readings obtained.

The *total chlorine*, existing as chlorides, cannot be ascertained in vinegar by direct precipitation with silver nitrate. For a correct analysis, 50 c.c. of the vinegar should be neutralised with alkali, evaporated to dryness, the residue ignited, dissolved in water, and the aqueous solution precipitated with excess of calcium sulphate or nitrate to remove phosphates. The filtrate from this precipitate

may be precipitated by, or titrated with, a solution of silver nitrate.

The sulphuric acid and sulphates may be precipitated by the direct addition of barium chloride to the diluted vinegar, but the figure has little value.

*Free sulphuric acid*, as distinguished from sulphates, may be estimated with considerable accuracy by evaporating 100 c.c. of the vinegar to a small bulk and then adding to the cold concentrated liquid 4 or 5 times its volume of alcohol. Sulphates are precipitated, whilst free sulphuric acid remains in solution. The filtered liquid is diluted, the alcohol boiled off and the sulphuric acid precipitated with barium chloride. The precipitate is filtered off, washed, dried, ignited and weighed. Its weight, multiplied by 0.4206, gives the weight of sulphuric acid in the quantity of vinegar taken. In a vinegar free from chlorides this process gives results in accordance with Hehner's process, but in their presence the mineral acid found is deficient by the amount of sulphuric acid required to decompose the chlorides. This difficulty may be obviated by treating the vinegar with excess of silver sulphate solution before evaporation, by which treatment any free hydrochloric is also estimated as sulphuric acid.

An ingenious method of detecting *free sulphuric acid* in vinegar and wine has been described by Casali. 20 grm. weight of the sample is ground up in a mortar with about 80 grm. of finely powdered porcelain (previously treated with hydrochloric acid to remove every trace of free alkali), so that the mixture is not moist to the touch. The whole is then ground up with 50 c.c. of ether (previously agitated with magnesia and water to neutralise any trace of acid), filtered and washed with ether. The filtrate is then shaken with a little distilled water, the ether distilled off and the residue precipitated with barium chloride; 0.0005 grm. of free sulphuric acid can be readily detected by this method.

A very simple and apparently reliable method of detecting *free mineral acids* in vinegar has been described by A. Ashby.<sup>1</sup> A solution of logwood is prepared by pouring 100 c.c. of boiling water on about 2 grm. of fresh logwood chips, and then allowing the decoction to stand for a few hours. Separate drops of this solution are spotted

<sup>1</sup>This method is one of those adopted by the Association of Official Agricultural Chemists.

on the surface of a flat porcelain dish or on the cover of a porcelain crucible, and evaporated to dryness over a beaker of boiling water. To each spot a drop of the suspected sample (previously concentrated, if thought desirable) should be added, and the heating continued till the liquid has evaporated. If the vinegar is pure the residue will be found to have a bright yellow colour, but in presence of a very small proportion of mineral acid the residue assumes a red colour.

If the proportion of mineral acid is very small, the red colour is destroyed on adding water to the residue, but is restored on evaporating, except in the case of nitric acid.

*Tartaric acid* in vinegar may be detected as described under wine vinegar, of which it is a normal constituent.

*Oxalic acid* may be detected by evaporating 20 c.c. of the vinegar to a small bulk, diluting the residue with water, and adding calcium-acetate solution or a mixture of ammonium acetate and calcium chloride. Any oxalic acid causes the formation of white calcium oxalate.

*Arsenic* has been occasionally met with in vinegar, and may be introduced in the malt or by the use of impure hydrochloric or sulphuric acid for inverting grain. It may readily be detected and estimated by Marsh's test (Analyst, 1902, 27, 48).

*Lead and copper* may be detected as described on page 651.

*Zinc* is occasionally present in vinegar. It may be detected by boiling down the vinegar to dryness with nitric acid, dissolving the residue in acidified water, passing hydrogen sulphide, filtering from any precipitate and then adding ammonium acetate, when white zinc sulphide will be thrown down if the metal is present. A less satisfactory method is to neutralise the greater part of the free acid in the original vinegar by ammonia, and then at once passing in hydrogen sulphide.

*Cayenne pepper and ginger* are said to be added to vinegar to confer pungency. They may be detected by neutralising the concentrated vinegar with sodium carbonate and *tasting* the liquid.

Flies and so-called "vinegar mites" and "eels" (*Leptodera oxophila*) are often found in vinegar which has been exposed to the air. They are readily detected by the microscope, and may be destroyed by raising the temperature of the liquid to 100°.

**Analysis of Commercial Vinegar.**—The following is a summary of some of the processes adopted and published in September, 1920, by the *Association of Official Agricultural Chemists*.

*Microscopic examination* should be made of the sediment, but the sample for chemical examination should be filtered and tested as soon as possible. All results are expressed by weight, but cubic centimeters are regarded as equivalent to grams in routine testing. The sp. gr. is determined by means of a pycnometer at  $20^{\circ}/4^{\circ}$ .

*Alcohol* (official).—Measure 100 c.c. of the sample into a round-bottomed distillation flask. Make faintly alkaline with saturated sodium hydroxide solution, add a small piece of paraffin, distil almost 50 c.c., make up to 50 c.c. at the temperature of the sample and determine the specific gravity at  $20^{\circ}/4^{\circ}$  by means of a pycnometer. Obtain the alcohol percentage from tables, noting that the alcoholic strength is twice that of the original vinegar.

*Total Solids* (official).—Measure 10 c.c. of the sample into a tared flat-bottomed platinum dish of 50 mm. bottom diameter, evaporate on a boiling water-bath for 30 minutes, and dry for exactly 2.5 hours in a water oven at the temperature of boiling water. Cool in a desiccator and weigh.

*Ash* (official).—Measure 25 c.c. of the vinegar into a tared platinum dish, evaporate to dryness on a steam bath, heat in a muffle at low heat to expel inflammable gases, treat the charred portion with a few cubic centimeters of water, and evaporate to dryness on a water-bath; replace in the muffle at a low redness for 15 minutes, and continue the alternate evaporation and heating, until a white or grey ash is obtained, at no time exceeding a dull red heat; cool in a desiccator and weigh.

*Solubility and Alkalinity of the Ash* (official).—Add water to the ash obtained as above, heat nearly to boiling, filter through an ash-less filter and wash with hot water until the combined filtrate and washings measure about 60 c.c. Return the filter and contents to the platinum dish, ignite carefully, cool and weigh. Cool the filtrate and titrate with N/10 hydrochloric acid, using Methyl Orange as an indicator.

*Phosphoric Acid*.—This is ascertained in both the water-soluble and insoluble portions of the ash by the standard methods of fertiliser analysis.

*Total Acids* (official).—Dilute 10 c.c. of the sample with recently boiled and cooled water until it appears very slightly coloured, and titrate with N/2 alkali, using phenolphthalein as an indicator. 1 c.c. of N/2 is equivalent to 0.03 grm. of acetic acid.

*Fixed Acids* (official).—Measure 10 c.c. of the vinegar into a 200 c.c. porcelain casserole, evaporate just to dryness, add 5 to 10 c.c. of water, and again evaporate; repeat until at least five evaporations have taken place. Add about 200 c.c. of recently boiled and cooled water and titrate with N/10 alkali, using phenolphthalein as an indicator. 1 c.c. of N/10 alkali is equivalent to 0.0067 grm. of malic acid.

*Volatile Acids* (official).—Subtract the fixed acids calculated as acetic acid from the total acids.

*Tartaric Acid and Tartrates*. Qualitative (official).—Evaporate 50 c.c. of the vinegar in a porcelain dish to a volume of about 10 c.c., filter into a test-tube, add 1 c.c. of 25% calcium chloride solution and 2 c.c. of 50% ammonium acetate solution and allow to stand overnight. In the presence of tartaric acid a deposit of calcium tartrate is formed the crystals of which may be identified under the microscope by their characteristic form.

*Total Tartaric Acid* (official).—Evaporate 200 c.c. of the sample to a syrupy consistency to remove excess of acetic acid, dilute to the original volume with water in a volumetric flask, determine the acidity as for total acids and determine tartaric acid in a 100 c.c. aliquot as directed under "Tartaric Acid in Wine" (page 235).

*Mineral Acids*.—For the logwood method see page 666. Another method is as follows: 5 c.c. of the sample are diluted with 10 c.c. of water, then a few drops of a solution of Methyl-Violet (1 part of colour to 10,000 of water) are added. If the liquid becomes blue or green, mineral acid is present.

The amount of mineral acids may also be estimated as follows (tentative): To a measured amount of the sample add a measured excess of standard alkali, evaporate to dryness, incinerate and titrate the ash with standard acid, using Methyl Orange as an indicator. The difference between the number of cubic centimeters of alkali first added and the number of cubic centimetres of acid required to titrate the ash represents the free mineral acid present.

*Caramel*.—L. Ronnet (*Ann. Fals.*, 1912, 5, 517) gives the following method for detecting caramel in vinegar. 50 c.c. of the vinegar

are mixed with an excess of calcium carbonate and evaporated to dryness. The mass is extracted with 20 c.c. of ether, the ethereal solution is filtered and the filtrate received in a test-tube containing 10 c.c. of resorcinol reagent (1 grm. of resorcinol dissolved in 100 c.c. of hydrochloric acid of sp. gr. 1.125). If caramel is present in the vinegar, a red zone is formed at the junction of the two liquids. Methods which depend on heating the vinegar with albumin and extracting the evaporated solution with ether are untrustworthy, as traces of furfural compounds (on which the colouration with the resorcinol reagent depends) may be formed during the evaporation, should the vinegar contain sugars.

Miss A. Anderson (*J. Ind. Eng. Chem.*, 1914, 6, 214) obtained positive tests for furfural by the aniline acetate method in 19 out of 28 samples of *pure* cider vinegar. She also obtained indications of caramel by using Fiehe's resorcinol reagent when no caramel had been added. Hence caution must be exercised in the interpretation of results. These observations emphasize the necessity, of modifying the test in some such manner as suggested by Ronnet owing to the production of furfural derivatives from sugars.

Other methods of analysing vinegars and interpreting the results of analyses are given by C. A. Mitchell in *Vinegar: Its Manufacture and Examination*, 1916.

**Acetates.**—Many of these are extensively used in the arts and medicine. Their analytical characters and the general methods adopted for their *analysis* have been, in great measure, already described. The following observations, therefore, have reference chiefly to the detection of impurities and adulterations in commercial acetates. Sections treating of acetic esters and acetates containing nitrogenous bases will be found in other parts of this work.

**Potassium acetate**,  $\text{KC}_2\text{H}_3\text{O}_2$ .—This exists in some vegetable secretions. It is deliquescent, very soluble in water and alcohol and neutral to litmus. It fuses at incipient redness, and at a higher temperature decomposes, leaving potassium carbonate. The amount of acetate present in commercial samples may be ascertained by the general methods given on page 673.

**Commercial potassium acetate** is liable to contain sulphates, chlorides and carbonates; iron, lead, copper and zinc; arsenic is occasionally present. It is sometimes intentionally adulterated,

calcium acetate and potassium sulphate, potassium tartrate or potassium carbonate being employed.

Potassium acetate being soluble in alcohol, any admixture of *sulphates*, *tartrates* or *carbonates* may be detected and estimated by treatment with that solvent. *Carbonate* is indicated more precisely by alkaline reaction; precipitation by calcium chloride; power of decolorising iodised starch; and effervescence on adding an acid.

**Sodium acetate**,  $\text{NaC}_2\text{H}_3\text{O}_2$ , closely resembles the potassium salt, but crystallises with 3 molecules of water. It is liable to contain the same foreign matters as potassium acetate. Crude sodium acetate often contains tarry matters derived from the pyroligneous acid employed in its preparation. Its supersaturated solution has been used for filling foot-warmers.

**Ammonium acetate**,  $(\text{NH}_4)\text{C}_2\text{H}_3\text{O}_2$ .—This salt is generally met with in solution, but may be obtained in the solid state, when it is apt to contain acetamide.

Ammonium acetate is liable to contain much the same impurities as the potassium salt, and may be examined in a similar manner. It should be wholly volatile on ignition.

**Calcium acetate**,  $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$ .—This crystallises with difficulty in prismatic needles containing 1 molecule of water. It is decomposed by heat into acetone and calcium carbonate.

Calcium acetate should be completely soluble in water and in proof spirit. An insoluble residue may consist of calcium sulphate or carbonate. The solution should give no precipitate with silver nitrate or barium chloride. Potassium ferrocyanide colours the solution blue if the sample contains iron, and brown if copper is present.

**Analysis of "Acetate of Lime."**—This is the commercial name for calcium acetate obtained from crude pyroligneous acid. It is often very impure, containing tarry matter; calcium hydroxide, carbonate and sulphate and salts of the homologues of acetic acid. Its analysis is of importance and somewhat difficult. If the salt is ignited, and the amount of acetic acid calculated from the weight of the residual calcium carbonate or from the amount of normal acid the residue will neutralise, very erroneous results may be obtained.

Calcium formate has been found in crude acetate, the proportion sometimes reaching 4 or 5%. When operating on the large scale,

the presence of formates is unmistakable. On crystallising out sodium acetate as completely as possible, a dense syrupy liquid is left which contains sodium formate, reduces silver and mercuric salts, and evolves carbon monoxide when treated with excess of sulphuric acid.

A method of analysis, much used in the neighborhood of Manchester, England, has been described by H. Grimshaw: 10 grm. of the sample of crude acetate are dissolved in boiling water, and 20 grm. of crystallised sodium sulphate added. The liquid is raised to boiling, cooled, diluted to 250 c.c., and allowed to stand for 12 hours. The calcium will then have separated as crystalline calcium sulphate. The liquid is filtered, the precipitate washed with hot water, and the filtrate made up to 500 c.c. 50 c.c. of this solution (= 1 grm. of the sample) should then be evaporated to dryness at 100°, and somewhat further dried in an air-bath. The residue is ignited at a red heat over a good Bunsen burner for half an hour, allowed to cool, and treated with 10 c.c. of N/1 hydrochloric acid, using a cover to avoid loss. The solution is boiled well to drive off carbon dioxide, filtered, the residual carbon washed, and the filtrate titrated with N/10 sodium hydroxide, using Methyl Orange or litmus as an indicator. Each cubic centimeter of normal acid found to have been neutralised by the ash represents 0.060 grm. of acetic acid ( $C_2H_3O_2$ ), or 0.079 grm. of calcium acetate, in the liquid (= 1 grm. of the sample) evaporated. Great care is requisite in conducting the titration, as a very small difference in the volume of alkali required makes a sensible change in the result. The portion of the sample taken for the analysis should be finely powdered, and if the solution in water be appreciably acid it should be cautiously neutralised with sodium hydroxide before adding the sodium sulphate. Grimshaw found this process to give results ranging from close agreement to about 2% in excess to those obtained from the same samples by distillation with phosphoric acid. The results are not vitiated by the presence of calcium carbonate or other insoluble calcium compounds in the sample.

Allen found a tendency to incomplete decomposition of the acetate if too low a temperature is employed. He suggested that a volume of solution representing 5 grm. of the sample be evaporated, and ignited at a moderate red heat in a muffle, subsequently moistening the ash with hydrogen peroxide to oxidise any sulphides which may have been formed.

The distillation process given in the preceding edition of this work was communicated by Stillwell and Gladding, being an improvement on the process published previously by them. A further communication by Stillwell will be found in *J. Soc. Chem. Ind.*, 1904, **23**, 305. The following process, essentially the same, is given by H. C. Sherman (Methods of Organic Analysis) as now in general use. For an extended account of methods of assaying commercial calcium acetate, see a paper by Grosvenor in *J. Soc. Chem. Ind.*, 1904, **23**, 530.

A 300 c.c. flask is fixed at an angle of about 60 degrees from the perpendicular and connected with a nearly vertical condenser whilst another tube passing through the stopper of the flask provides for the introduction of water, drop by drop, during distillation. The flow of water should be under complete control. The weighed material (2 grm.) finely ground (to pass an 80 mesh screen) is transferred to the flask, 15 c.c. of 50% phosphoric acid and 25 c.c. of water added, taking care that the water washes down any powder or acid that is in the neck of the flask. The apparatus is connected and the distillate collected in a receiver containing 50 c.c. of water. During the process, the volume of the liquid should be kept at 40 c.c., as near as may be, by admitting water free from carbonic acid, adding it so that the drops fall on the side of the flask and not directly into the distilling liquid. It is stated that it is advantageous to use water containing a little phosphoric acid. The operation is continued until the distillate is no longer acid, which usually requires about 90 minutes. The distillate is titrated with fresh standard alkali.

This modification of the usual methods avoids the danger of phosphoric acid being carried over mechanically. In grinding the sample care must be taken not to lose moisture. It is recommended to evaporate the titrated distillate and apply the usual qualitative test for phosphoric acid to make sure that no appreciable amount of this has been carried over. The distillate, of course, contains all the other volatile acids present of which the salts are present in the sample.

The phosphoric acid employed for the distillation must be free from nitric acid, which if present may be eliminated by adding a little ammonia, and heating the acid to fusion in platinum. If either the phosphoric acid or the sample itself contains chlorides, some silver sulphate must be added to the contents of the retort. Oxalic acid may be substituted for the phosphoric acid, the solution being filtered from the precipitated calcium oxalate before intro-

duction into the retort. Hydrochloric acid may be used instead, provided that the amount which passes into the distillate be estimated and subtracted from the total acidity as deduced from the titration. Sulphuric acid should not be used, as its reaction on the tarry matters occasions the formation of sulphurous acid, which increases the acidity of the distillate.

Gladding has described (*J. Ind. Eng. Chem.*, 1909, **1**, 250) a modification of the process as carried out in his laboratory with satisfaction. The apparatus shown in Fig. 104 is used. 2 grm. of the sample and 30 c.c. of water are introduced into *A* (about 300 c.c. capacity); 10 c.c. of phosphoric acid (sp. gr. 1.7) are added and the liquid boiled gently for about 90 minutes, whilst the volume is kept at 50 c.c. The distillate, condensed in *C*, is received in *B*, which contains 30 c.c. of standard alkali. At the end of 90 minutes, distillation the contents of *B* are titrated. The distillation should be continued until the distillate is neutral. Phenolphthalein is used as an indicator. A blank experiment should be made. The volume of liquid in *A* is kept at 50 c.c. by adding water drop by drop through the tube *H*. Gladding refers to a paper by Fresenius and Grünhut (*Zeitsch. anal. Chem.*, 1908, 597) in which these authors disapprove of his method, but he shows that they did not subject it to proper comparison and that the method is trustworthy. When pure calcium acetate is analysed by either of the foregoing methods accurate results may be obtained, but

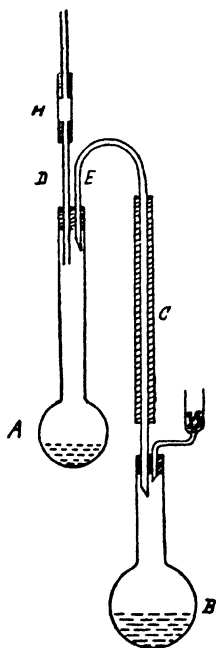


FIG. 104.

when commercial samples are examined the errors may sometimes become serious. On the whole, the method of distillation with phosphoric acid is the most accurate, but, unless carefully performed, the results are liable to be below the truth, from incomplete volatilisation of the acetic acid, whilst on the other hand, they may be excessive if nitric or other volatile acid be present in the phosphoric acid used.<sup>1</sup>

<sup>1</sup> The following results, reported by Allen from the same sample of "acetate of lime" by different methods, show the nature and direction of the errors to which the various processes are liable:

	ACETIC ACID, PER CENT.
By distillation with phosphoric acid and titration of distillate.....	47.4
By distillation with phosphoric acid and titration of distillate.....	48.0
By distillation with sulphuric acid and titration of distillate.....	48.6
By distillation with oxalic acid and titration of distillate.....	48.3
By distillation with oxalic acid and titration of distillate.....	48.4
By Fresenius' method.....	53.4
By Fresenius' method.....	53.2
By ignition and weighing the calcium carbonate.....	53.2
By ignition and titration of residue.....	53.2
By ignition and titration of residue.....	53.8
By ignition and titration of residue.....	53.0
By boiling with sodium carbonate, and titrating filtrate.....	56.4
By boiling with sodium carbonate, and titrating filtrate.....	56.4
By boiling with sodium carbonate, and titrating filtrate.....	57.6

Improvements in the manufacture of calcium acetate render the discrepancies resulting from the employment of different methods of assay less striking than formerly.

Duchemin and Criquebœuf (*Bull. Ass. Chim. Dist*, 24, 1216) have proposed a method for the volumetric estimation of combined acetic acid in acetates based on the fact that Methyl Violet turns blue in presence of free sulphuric acid (between N/10 and N/100), but is unaffected by free acetic, propionic or butyric acids and only slightly by free formic acid. If the solution contains a free mineral acid or alkali, it must first be neutralised, using phenolphthalein as an indicator. If it contains coloured metallic ions or such as give a precipitate with sulphuric acid, they must be precipitated. If the solution is coloured with empyreumatic products, the colour test is carried out on a porcelain plate. This is also done when titrating formates, as formic acid is not without action on Methyl Violet. In this case a weak solution of formic acid containing a sulphate is used for comparison. Insoluble basic acetates may be dissolved in a known quantity of acetic acid and then tested in the same way.

**Magnesium acetate.**—Basic magnesium acetate has been recommended as an antiseptic.

**Aluminum acetate.**—This salt is employed in solution by calico-printers under the name of "red liquor." It is usually prepared by precipitating a solution of alum or aluminum sulphate by means of calcium or lead acetate, and filtering or syphoning off from the precipitated calcium or lead sulphate. A better method, designed to give more accurate results, consists in precipitating aluminum hydroxide from a solution of aluminum sulphate by means of the calculated quantity of sodium carbonate. The precipitate is washed by decantation and dissolved in the desired quantity of acetic acid. A product of any desired basicity can be obtained. When prepared by means of alum, the product necessarily contains potassium sulphate or ammonium sulphate (according to the kind of alum used),

and, as an excess of the precipitant should be avoided, aluminum sulphate is always to be expected. Owing to calcium sulphate being somewhat soluble in water, it will be met with in red liquors prepared with calcium acetate. Such red liquor is inferior to that prepared by lead acetate. Good red liquor contains the equivalent of from 3 to 5% of alumina, and twice that proportion of acetic acid, and has a gravity of 1.120, but it is sometimes met with as low as 1.087. Sodium carbonate is often added to red liquor to neutralise excess of acid.

**Iron acetates.**—Both ferrous and ferric acetates are employed in the arts. A crude variety of iron acetate is extensively manufactured by dissolving iron in pyroligneous acid.

**Pyrolignite of Iron, Iron Liquor or Black Liquor.**—For use by calico-printers a liquid consisting chiefly of a solution of ferrous acetate, but always containing more or less ferric acetate, is prepared by acting on scrap-iron by crude pyroligneous acid of 1.035 to 1.040 sp. gr. A purified acid gives less satisfactory results. The product, which is a deep black liquid, has a gravity of 1.085 to 1.090, and is concentrated by boiling till it is about 1.120, when it contains about 10% of iron. It is then ready for use, and is known as "printers' iron liquor." Much iron liquor is now made as high as 1.140. For use by dyers, the liquid is not concentrated by evaporation, but the gravity is raised by the addition of ferrous sulphate (copperas), by which a more suitable product is said to be obtained than is yielded by iron acetate alone. As a 5% solution of crystallised ferrous sulphate has a gravity of 1.026, the addition of 0.5 lb. of copperas to the gallon of "black liquor" will raise it from 1.085 to 1.111. As much as 124 gm. of ferrous sulphate per 1000 c.c. has been met with in iron liquor. The sulphate may also result from the addition of sulphuric acid to the pyroligneous acid employed for dissolving the scrap-iron. Iron sulphate may be detected and estimated by treating the diluted black liquor with barium chloride. 233 parts of the precipitate represent 278 parts of crystallised ferrous sulphate. Black liquor is frequently adulterated with common salt, a 5% solution of which has a gravity of 1.036. It may be detected and estimated by adding nitric acid and treating the diluted liquor with silver nitrate. Iron chlorides may also be present owing to the addition of hydrochloric acid to the pyroligneous acid. Hence the chlorine must not be assumed to exist as common salt without further

examination. This is best effected by heating the liquid with nitric acid, adding barium nitrate to separate the sulphates, precipitating the iron and excess of barium by ammonium hydroxide and carbonate, evaporating the filtrate to dryness, and igniting the residue, when any common salt will remain. *Tannin* is stated to be occasionally added to iron liquor.

**Ferrous acetate** is sometimes made by decomposing a solution of ferrous sulphate with calcium acetate. The liquor has usually a gravity of 1.11, and contains calcium sulphate.

**Ferric acetate** is sometimes preferred by dyers and printers to the ferrous salt. It is occasionally prepared by decomposing iron-alum or ferric sulphate by lead acetate. The product must be free from excess of the lead salt, and, for some purposes, excess of ferric sulphate must be avoided.

**Tincture of ferric acetate** may be prepared by mixing alcoholic solutions of potassium acetate and ferric sulphate, and filtering from the precipitated potassium sulphate.

**Lead acetates.**—These include neutral acetate,  $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$ , often called "sugar of lead," and several basic- or oxyacetates, all of which are more or less soluble in water, the solutions possessing an alkaline reaction and giving a precipitate of lead carbonate under the action of carbon dioxide. A solution of neutral lead acetate is but little affected by carbon dioxide. By suspending basic acetate in water and passing carbon dioxide through the liquid as long as it has an alkaline reaction, the lead is separated as an insoluble carbonate, and may be filtered off, washed, ignited in porcelain (apart from the filter) till bright yellow when cold, and weighed as lead monoxide. The lead remaining in permanent solution exists as *acetate*, and may be ascertained by precipitation as sulphate or chromate.

A better and simpler method for detecting basic acetate in a sample is to dissolve it in recently boiled water, filter, and then add to the clear solution an equal volume of a 1% solution of mercuric chloride. A white precipitate proves the presence of basic acetate. The analysis may also be conducted by methods given on page 674.

Fresenius recommends the following indirect method for the analysis of pyrolignite and lead acetate: 10 grm. of the sample are dissolved in water in a flask holding 500 c.c., 60 c.c. of normal sulphuric

acid are added, and the water up to the mark. An extra 1.3 c.c. of water is added to compensate for the volume of the precipitated lead sulphate. The flask is closed, well shaken, and the liquid allowed to settle. 100 c.c. of the clear liquid are taken out, treated with barium chloride, and the precipitate collected, washed, ignited and weighed. Its weight, multiplied by 0.4206, is subtracted from 0.588 grm. (the weight of acid added to each 100 c.c. of the liquid). The remainder, multiplied by 113.7, gives the percentage of lead monoxide in the sample. Another 100 c.c. of the clear liquid are drawn off and titrated with N/1 sodium hydroxide, using litmus as an indicator. Multiply the number of c.c. of alkali used by 0.060, subtract from this the previously obtained weight of barium sulphate multiplied by 0.515 (= the free sulphuric acid expressed in terms of acetic acid), and the remainder, multiplied by 50, will be the percentage of acetic acid in the sample.

**Basic lead acetate.**—Solutions of basic lead acetate have been long used in medicine and formulas for their preparation are given in pharmacopœias. A solid basic acetate and a solution are much used as clarifying agents in saccharimetry. For the method of preparing such a solution see page 374.

**Cupric acetates.**—Several of these salts are known and extensively used in the arts. They are prepared by the action of acetic acid on copper oxide or carbonate or upon metallic copper with access of air. The neutral acetate is freely soluble in water, but several basic acetates exist. They are of different shades of colour, and are known as blue and green verdigris.

**Verdigris** of good quality is dry, soluble in dilute acetic acid, sulphuric acid or ammonium hydroxide. It should contain not more than 4% of impurities. A good sample will correspond to about the following composition: Cupric oxide, 43.5; acetic anhydride, 29.3; water, 25.2; and impurities, 2.0. It is frequently adulterated. Sand, clay, pumice and chalk; barium, calcium and copper sulphates; and iron and zinc compounds, are sometimes present. Zinc in verdigris is usually due to the use of sheets of brass instead of copper for corrosion by acetic acid.

On dissolving the sample in dilute hydrochloric acid, any *sand*, *clay*, *pumice*, or *barium sulphate* will be left insoluble, and may be collected and weighed. (About 3% of insoluble matter is allowable in verdigris. If the residue amounts to 6% the sample is inferior.

*Calcium sulphate* in large proportion may be left partially in the insoluble residue.) If the sample effervesced on addition of acid, a *carbonate* is present, though it may be that of copper. From a measured portion of the solution in acid the *sulphates* may be precipitated by barium chloride, the precipitate collected and weighed.

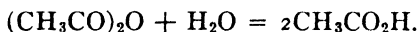
For the detection of the *metals*, the sample should be ignited, the residue dissolved in hydrochloric acid, and the copper precipitated from the diluted liquid by a current of hydrogen sulphide. In the filtrate the excess of hydrogen sulphide is destroyed by bromine water, the liquid nearly neutralised with ammonium acetate, and then boiled with ammonium acetate. The precipitate, when washed and ignited, is ferric oxide. The filtrate from the iron precipitate is treated with hydrogen sulphide and any white zinc sulphide filtered off, carefully roasted and weighed as oxide. From the filtrate, the calcium is precipitated by ammonium oxalate. The precipitate yields calcium carbonate on gentle ignition, the weight being equal to the chalk in the quantity of the sample taken. The calcium may be estimated more readily, but less accurately, by dissolving the sample in hydrochloric acid, precipitating the iron with bromine and ammonium hydroxide, and then at once treating the filtrate with ammonium oxalate. Of course, it does not follow that all the calcium found exists as chalk, unless sulphates are absent.

**Acetic anhydride.**—Acetic anhydride is a colourless, mobile liquid of pungent odour. Sp. gr. at  $20^{\circ}$  1.073, b. p.  $137^{\circ}$ . It has found extended use in recent years for the manufacture of cellulose acetate, acetyl-salicylic acid (aspirin), and acetylations generally. The commercial article is not pure anhydride, but contains varying amounts of acetic acid, and is sold on the percentage of anhydride, which ordinarily amounts to 70 to 90%. Depending on the source of the raw materials, it may also contain higher fatty acids and anhydrides, as well as sulphates and chlorides. It is usually made from anhydrous sodium acetate and sulphur chloride, or sometimes phosphorus oxychloride or phosgene. Weak acetic anhydride may be concentrated by distillation over anhydrous sodium acetate. It is insoluble in water, but is slowly hydrolyzed by it to acetic acid.

The percentage of anhydride can not be estimated directly. The procedures generally used for its analysis are discussed by Rad-

cliffe and Medofski (*J. Soc. Chem. Ind.*, 1917, **36**, 628). They depend on the hydrolysis of the anhydride by means of water or alkali and titration of the resulting acetic acid, or on the reaction of the anhydride with aniline and titration of the remaining acetic acid. In both cases the assumption is made that the mixture contains nothing but acetic anhydride and acetic acid, which is not true in the case of most commercial anhydrides. A combination of the two methods as given by the British Expert Committee's "Report on the Analysis of Crude Glycerin, 1911" (*Analyst*, 1911, **36**, 314) gives more accurate results of the value of an anhydride, although the numerical figure may not be absolutely correct. The following, which is a modification of the method just mentioned, is largely used in the United States.

A 2 c.c. sample of the anhydride is run from a burette into a straight-side, glass-stoppered weighing bottle and weighed. The weighing bottle and contents are then dropped into a 300 c.c. Erlenmeyer flask containing 50 c.c. of N/1 sodium hydroxide solution and 50 c.c. of water. The stopper of the bottle is removed as the bottle is dropped into the flask. The mixture is allowed to stand for 30 to 45 minutes at room temperature, with occasional shaking. It is then titrated to the end point with N/5 hydrochloric or sulphuric acid, using phenolphthalein as an indicator. From the figure obtained, the number of c.c. of normal alkali required to neutralise 100 grm. of the hydrolysed acetic anhydride is calculated (A).



About 20 c.c. of perfectly dry and *freshly distilled* aniline are run into a 50 c.c. weighing bottle and weighed. 2 c.c. of anhydride are then added, drop by drop, from the burette, the aniline being swirled slightly during the operation to prevent overheating. When the anhydride has all been added, the bottle is stoppered and set aside until it reaches room temperature, when it is weighed again in order to determine the weight of anhydride added. The contents of the weighing bottle are next washed into a 500 c.c. Erlenmeyer with 100 c.c. of 50% neutral alcohol, followed by an additional rinsing with distilled water. The solution is now titrated with N/1 alkali. From the result, the number of c.c. of N/1 alkali required to neutralise the acetic acid in 100 grm. of the sample is calculated (B).



The result (*A*) is, therefore, a measure of the sum of the acetic acid present as such and of that obtained by hydrolysis. Result (*B*) is a measure of the sum of the acetic acid present as such and one-half of that present as anhydride. If we subtract (*B*) from (*A*), we obtain the number of c.c. of normal alkali corresponding to one-half of the acetic anhydride in 100 grm. of sample. By multiplying this difference by 0.102, the percentage of anhydride is obtained.

This combination method is more accurate than either the titration or the aniline method alone, because impurities that tend to give low results in the former method generally tend to give high results in the latter, and vice versa.

Sulphuric and hydrochloric acids are estimated in the usual manner after hydrolysis. It is generally specified that they must not be present in greater amounts than 0.05% calculated as sulphuric acid or chlorine respectively.

### HOMOLOGUES OF ACETIC ACID. Lower Fatty Acids.

Acetic acid is the most important and best known of the homologous series called "the fatty acids." These acids have the general formula  $C_nH_{2n}O_2$ . The lower members of the series are volatile liquids closely resembling acetic acid. The higher members of the series are insoluble in water, not volatile without decomposition, and solid at ordinary temperatures. Many fatty acids are known, but the greater number are of very limited importance.

The higher members of the fatty acid series are almost exclusively obtained by the saponification of the fixed oils, fats and waxes, and such of them as require description will be considered in the section treating of these substances. The present article is limited to a consideration of the lower members of the series, sensibly volatile or soluble in water, and hence liable to occur under the same circumstances as acetic acid.

With the exception of the first three, all the members of the acetic series of acids are capable of isomeric modification. The number of such modifications increases rapidly with the number of carbon atoms in the molecules, and many have been obtained.

The following table gives the names of the normal and iso-acids of the acetic series up to the member with 7 carbon atoms. Above caproic acid the modifications have been imperfectly differentiated.

A table of the still higher members of the series will be given in the section on "Saponification."

From this table it will be observed that the b. p. of the normal fatty acids show a tolerably regular increase of  $18^{\circ}$  to  $22^{\circ}$  for each increment of  $\text{CH}_2$  in the formula. The iso-acid in each case boils at a lower temperature than the normal and has also lower sp. gr. The sp. gr. and solubility of the fatty acids, as also the solubility of many of their salts, decrease with an increase in the molecular weight. The esters of the fatty acids similarly diminish in solubility and volatility with each increase in the number of carbon atoms.

Empirical formula	Name	Constitutional formula	B. p. ° C.	Sp. Gr. at 0° C.	Solubility in water
$\text{CH}_2\text{O}_2$	Formic acid	$\text{H.COOH}$	100		Miscible in all proportions
$\text{C}_2\text{H}_4\text{O}_2$	Acetic acid	$\text{CH}_3\text{COOH}$	110		Do
$\text{C}_3\text{H}_6\text{O}_2$	Propionic acid	$\text{CH}_3\text{CH}_2\text{COOH}$	140	1.016	Do
$\text{C}_4\text{H}_8\text{O}_2$	Normal butyric acid	$\text{CH}_3(\text{CH}_2)_2\text{COOH}$	163	9817	Do
	Iso-butyric acid; or dimethacetic acid	$\text{CH}(\text{CH}_3)_2\text{COOH}$	154	9598	Soluble
$\text{C}_5\text{H}_{10}\text{O}_2$	Normal pantoic or valeric acid	$[\text{CH}_3(\text{CH}_2)_3\text{COOH}]$	185	9577	Sparingly (1 in 30)
	Iso-pantoic acid; ordinary valeric acid; or iso-propionic acid	$\text{CH}(\text{CH}_3)_2\text{CH}_2\text{COOH}$	175	9536	Do
$\text{C}_6\text{H}_{12}\text{O}_2$	Normal caproic acid	$\text{CH}_3(\text{CH}_2)_4\text{COOH}$	205	9450	Nearly insoluble
	Iso-caproic acid	$\text{CH}(\text{CH}_3)_2(\text{CH}_2)_3\text{COOH}$	199	9310	Do
$\text{C}_7\text{H}_{14}\text{O}_2$	Normal heptanoic acid	$\text{CH}_3(\text{CH}_2)_5\text{COOH}$	224	9345	Almost insoluble
	Iso-heptanoic acid	$\text{CH}(\text{CH}_3)_2(\text{CH}_2)_4\text{COOH}$	215		Do

As a rule, the iso-acids present very close resemblances to the corresponding normal acids, their lower gravities and b. p. and greater susceptibility to oxidation being the most marked distinctions. In some cases, differences are observable in the solubility and crystallisability of the salts.

As a class, the lower members of the acetic acid series may be separated from most other organic acids (except lactic acid) by treating the aqueous solution with finely ground lead monoxide in quantity sufficient to render it slightly alkaline. On filtering, the lead salts of most organic acids will be left insoluble, whilst those of the acetic series will be found in the filtrate.

The separation of the lower acids of the acetic series from each other cannot usually be effected readily; the most satisfactory methods are based on the following principles:

The lowest members of the series are the most readily soluble in aqueous liquids, formic, acetic, propionic and normal butyric acid, being soluble in all proportions. All but formic and acetic acids are separated from their aqueous solutions by saturating the liquid with calcium chloride, when they rise in the form of oils. A more perfect separation from acetic and formic acids of the acids higher than valeric may be effected by shaking the acidified aqueous solution with ether, which dissolves the higher homologues together with more or less of the lower homologues. On agitating the ethereal layer with a strong solution of calcium chloride the formic and acetic acid pass into the latter, and, by repeating the treatment, may be perfectly removed from the ether, with little or no loss of the higher homologues.

The lower members of the series are most active. Hence, if an amount of alkali insufficient for complete neutralisation is added to a solution containing the free acids, and the liquid is then distilled, the higher members of the series pass over in the free state, whilst the lower members remain behind as fixed salts.

If sodium hydroxide is added to a mixture of butyric and valeric acids in quantity insufficient to neutralise the whole, and the liquid is then distilled, the distillate will consist of pure valeric acid and the residue will contain mixed sodium butyrate and valerate; or else the distillate will contain the whole of the valeric acid and some butyric acid, and the residue will consist entirely of butyrate of sodium. In either case, a portion of one of the acids is obtained free from the other. In the first case, the residue of mixed valerate and butyrate may be treated with sufficient dilute sulphuric acid to neutralise half of the sodium hydroxide originally used, and the mixture redistilled, when a fresh quantity of valeric acid will be obtained, either pure or mixed with butyric acid according to the relative proportions of the two acids present in the original mixture. In the latter case, by partially neutralising the distillate with alkali, and again distilling, a further separation may be effected, and by repeating the operation in a judicious manner, two or even more of these volatile fatty acids may be separated fairly well from each other.

Although the foregoing method is well suited to the separation of normal butyric and valeric acids, the principle is wholly at fault when iso-valeric acid is in question, for this acid completely decomposes normal butyrates.

An approximate separation of the homologues higher than valeric acid can be effected by a fractional crystallisation of their barium salts. The following is the order in which the barium salts are deposited:

From aqueous solutions.

1. Barium caprate.
2. Barium pelargonate.
3. Barium caprylate.
4. Barium cœnanthylate.
5. Barium caproate.

From alcoholic solutions.

1. Barium caprylate.
2. Barium cœnanthylate.
3. Barium pelargonate and caprate.
4. Barium caproate.

The aqueous or alcoholic solution of the acid is neutralised with standard aqueous or alcoholic solution of sodium hydroxide (according as the crystallisation is to be effected from an aqueous or alcoholic solution), an amount of barium chloride equivalent to the alkali is next added, and the resultant liquid evaporated and allowed to deposit crystals. The crops of crystals from an aqueous solution may be washed with hot alcohol, the washings containing the salts in the reverse order of their deposition from alcoholic solution.

Another method of detecting and estimating acids of the acetic series, when in admixture with each other, is based on the different composition of their barium salts, the process being as follows: The free acids obtained by distillation are saturated with barium carbonate or by the cautious addition of baryta water (using phenolphthalein to indicate the point of neutrality), the latter method being preferable for the higher numbers of the series. In this way, neutral barium salts are formed, which may be obtained in the anhydrous state by evaporating off the water and drying the residue at  $130^{\circ}$ . These barium salts contain percentages of barium dependent on the atomic weights of the fatty acids present. On moistening the residue with sulphuric acid and then igniting it, an amount of barium sulphate is obtained proportional to the percentage of barium contained in the salt of the fatty acid present. Instead of weighing the barium sulphate, a standard solution of baryta water may be employed, and the weight of barium (or its equivalent of barium sulphate) calculated from the volume of solution employed. This method also serves as a useful check on the determination of the weight of barium sulphate. The following table shows the proportions of barium contained in, and of barium sulphate obtainable from, the barium salts of the lower acids of the acetic series:

Name of salt	Barium, %	Barium sulphate, %
Barium formate.....	70.25	119.47
Barium acetate.....	53.73	91.37
Barium propionate.....	48.41	82.13
Barium butyrate.....	44.05	74.91
Barium valerate.....	40.41	68.73
Barium caproate.....	37.33	63.48
Barium cœnanthylate.....	34.68	58.98
Barium caprylate.....	32.39	55.08
Barium pelargonate.....	30.38	51.66
Barium caprate.....	28.60	48.64

From this table it will be seen that the pure barium salts of the lower acids of the acetic acid can very readily be distinguished from each other by estimating the percentage of barium contained in them. In the case of mixtures of two acids the identity of which is established, the proportions in which the two are present may be calculated from the following formula, in which  $x$  is the percentage of barium salt of the lower fatty acid in the mixed barium salts obtained;  $P$ , the percentage of barium sulphate yielded by the mixed barium salts on treatment with sulphuric acids;  $B$ , the percentage of the same theoretically obtainable from the pure salt of the lower fatty acid; and  $b$ , the percentage of the same theoretically obtainable from the pure salt of the higher fatty acid. Then—

$$Bx = 100P + bx - 100b$$

For example, suppose a mixed barium salt known or assumed to consist of acetate and valerate to have yielded a precipitate of barium sulphate equivalent to 78.45% of the weight taken, when treated with sulphuric acid and ignited. Then, by the above formula,

$$91.37x = 7845 + 68.73x - 6873$$

therefore

$$22.64x = 972$$

and

$$x = 42.93.$$

Hence, the mixed barium salt consisted of 42.93 of barium acetate, and 57.07 of barium valerate. From these data and the weight of mixed barium salt found, the actual amounts of acetic and valeric acid may be calculated.

The above method was proposed by Dupré for approximately estimating the fusel oil in spirits. In this case the various alcohols

are first converted into the corresponding acids by oxidation with chromic-acid mixture.

It has been stated that butyric and valeric acids are extracted from a water solution by shaking with benzene, whilst formic and acetic remain in the water (see *Analyst*, 1908, 33, 133).

Duclaux (*Ann. Chim. Phys.*, [5], 1874, 2, 289) claimed to have established that each of the lower acids of the formic series has its own rate of distillation, whether alone or mixed with homologues. Several investigators have gone over this method and found it unsatisfactory. H. D. Richmond (*Analyst*, 1895, 20, 193, 217) examined it very carefully and decided that in the form given by Duclaux it is untrustworthy.

Richmond gives as the result of many experiments the following formula for the distillation of each acid, but it is not established that the formula will apply to any mixture.

$$100 - y = K \frac{(100 - x)^a}{100^{a-1}}$$

In this formula,  $x$  is the percentage of liquid distilled,  $y$  the percentage of acid distilled, and  $a$  and  $K$  are factors for each acid ascertained by experiment, as follows ( $K$  is practically negligible):

	$a$	$K$
Formic (Duclaux).....	0.4	1.00079
Acetic (Duclaux).....	0.667	
Propionic (Duclaux).....	1.111	1.000723
Butyric (Duclaux).....	2	1 (?)
Butyric (Wollny).....	2	1
Butyric (Richmond).....	2	1
Valeric (Duclaux).....	3	1 (?)
Caproic (Duclaux).....	4	0.003508
Caprylic (Duclaux).....	8 (?)	(?)

For an illustration of the practical application of this method, see a paper by Richmond in *Analyst*, 1908, 33. Further data on this method are found in *Analyst*, 1917, 42, 125-139. Landolt (*Chem. Ztg.*, 35, 687) has applied the method to artificial and natural fruit essences and has shown that the respective distillation curves have distinct differences. Dyer (*J. Biol. Chem.*, 1917, 28, 445) describes a somewhat similar method which depends upon the main-

tenance of the aqueous solution containing the acids to be distilled, at a constant volume of 150 c.c. throughout the course of the distillation. He also describes a number of colour tests for the recognition of the lower fatty acids.

**Formic Acid,  $\text{HCHO}_2$ .**—Formic acid is contained in the liquid obtained by distilling ants with water. The stings of some insects and plants probably contain it. It is usually prepared by distilling oxalic acid with glycerol. A formate is produced in the decomposition of chloroform or chloral by an alkali, by the reaction of carbon monoxide and alkalies, and of cyanogen gas or cyanides with water.

Formic acid is a colourless volatile liquid, of irritating pungent odour and very sour. It has a sp. gr. of 1.2211 at  $20^\circ$ , and boils at  $100^\circ$ . It produces intense irritation of the skin.

In general properties it resembles acetic acid, but it is stronger and more readily oxidised.

The *formates* mostly crystallise well and are all soluble in water. Heated with concentrated sulphuric acid they do not blacken, but evolve pure carbon monoxide, as a colourless gas burning with a pale blue flame. A neutral solution of formate gives the following reactions:

*Silver nitrate* gives, in concentrated solutions, white crystalline silver formate, which darkens on standing, and is reduced to metallic silver when warmed. If the liquid be too dilute to allow of a precipitate being formed, the reduction to metallic silver still occurs on heating, a mirror being frequently formed on the sides of the tube. In presence of ammonium hydroxide the reduction is retarded or prevented.

*Mercuric chloride* is reduced on heating, with production of white mercurous chloride or grey metallic mercury, according to the proportion of formate present. Acetates do not give this reaction, but acetates and chlorides of alkali metals retard or prevent the reduction. The reduction of mercuric formate on heating may be applied to the estimation of formic acid, and its separation from acetic acid may be approximately effected by boiling the solution of the free acids with yellow mercuric oxide until effervescence ceases. If formic acid only is present, the filtered liquid will be free from mercury. With a mixture of the two acids, the amount of mercury which passes into solution is equivalent to the acetic acid present. If the total acid present originally is determined by

standard alkali or other means, the quantity of formic acid may be found, or in presence of other acids forming soluble mercuric salts, the excess of mercuric oxide may be dissolved by dilute hydrochloric acid, and the residual metallic mercury weighed. This weight multiplied by 0.23 will give the weight of formic acid present.

In testing for formic acid in distillates it must be borne in mind that the distillation of sugars with acids produces formic acid.

It is also dangerous to apply either the silver nitrate or mercuric chloride tests for formic acid to distillates, inasmuch as other volatile substances will give a positive indication with these reagents. Consequently it is advisable to convert the formic acid into formaldehyde by means of magnesium and sulphuric acid and then apply a convenient test for formaldehyde.

*Chlorine*, bromine, chromic acid, permanganates and other powerful oxidising agents convert formic acid more or less readily into carbonic acid.

Joseph (*J. Soc. Chem. Ind.*, 29, 1189) proposes to make use of this action of bromine for the estimation of formic acid or formates. If formic acid or formates are present alone or with inorganic salts, the solution is exactly neutralised, boiled and bromine water added until the colour persists. The excess of bromine is then expelled by continued boiling and the liberated hydrobromic acid estimated by titrating with standard alkali. A blank must be run with approximately the same amount of water and excess of bromine, as bromine water boiled alone gives a small amount of hydrobromic acid. If acetates are present, the total combined bromine left after the expulsion of the free bromine is determined by Volhard's method. (See also Maeder, *Apoth. Ztg.*, 27, 746.)

When heated gently with *alcohol and sulphuric acid*, formates generate ethyl formate, having a fragrant odour of peach-kernels. With ferric chloride, formates react similarly to acetates. At a gentle heat, strong sulphuric acid evolves carbon monoxide from formic acid or a formate. Strong alkalies produce an oxalate.

Lead and magnesium formates are insoluble in alcohol, whilst the corresponding acetates are soluble. Hence, acetic may be separated from formic acid by saturating the free acids with a slight excess of calcined magnesia or lead carbonate, filtering, evaporating the filtrate to a small volume, and adding a large proportion of alcohol. Magnesium or lead formate is precipitated, whilst the corresponding

acetate remains in solution. The process may be modified by precipitating the alcoholic solution of the acids with an alcoholic solution of lead acetate, and washing the resultant precipitate with alcohol.

Formic acid may be detected by reduction to formaldehyde. Fenton and Sisson (*Proc. Cambridge Philos. Society*, 1907, **14**, 385) find that this is best accomplished by the action of magnesium in powder or ribbon. A few minutes suffice to produce sufficient formaldehyde for detection by the standard tests. Of course, the absence of formaldehyde must be first established. If it is present, it may be destroyed by pure potassium cyanide as described on page 99. The formic acid can be separated by distilling and the distillate tested.

In addition to the methods already indicated, formic acid may be estimated by titration with standard alkali or by decomposition in a carbonic acid apparatus by sulphuric acid and potassium dichromate, the amount of formic acid present being deduced from the weight of carbon dioxide evolved. 44 parts of carbon dioxide are equivalent to 46 parts of formic acid.

*Formic acid* and *sodium formate* are used as food preservatives. Woodman and Burwell (*Tech. Quart.*, 1908, **21**, 1), have devised the following method for detecting these substances in food.

50 grm. of the sample are mixed with 20 c.c. of 20% phosphoric acid solution, and distilled by means of atmospheric steam, the mixture being gently heated to avoid much condensation. A distillate of about 200 c.c. should be collected. Almost all the formic acid is thus obtained. The distillate is mixed with 2 c.c. of 30% acetic acid (free from formic) and 0.2 grm. calcium hydroxide in form of milk of lime. If the distillate is very acid more of the hydroxide may be needed. The solution is evaporated to small volume over a free flame, and then, on a steam-bath, to dryness. The evaporation should be carried as far as possible over the flame, as the boiling prevents the formation of a crust of calcium carbonate. The dry residue is put into a test-tube provided with a cork and delivery tube. The lower end of the delivery tube should dip into about 3 c.c. of water in a tube standing in cold water. The test-tube containing the dry residue is heated gradually to redness, or, at least until vapours are no longer produced. Formic acid or a formate present in the original material will give formaldehyde in the final distillate. W.

and B. use the fuchsin test (see p. 323). As a slight reaction is produced by the products of destructive distillation even in absence of formate, they use a colour standard prepared by mixing 8 c.c. of copper chloride solution (12 grm.  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  in 1000 c.c.) and 12.5 c.c. cobalt chloride solution (24 grm.  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  and 100 c.c. strong hydrochloric acid in 1000 c.c.) and diluting this mixture to 100 c.c. Many food products were tested by W. and B., and found not to give a colour greater than the standard, whilst 0.025 grm. of formic acid in 50 grm. of material gave a colour from 4 to 6 times as deep.

Bacon (*Bur. Chem. Circ.*, 74) bases a quantitative estimation of formic acid on the fact that in neutral or faintly acid solutions it is the only substance which reduces platinum chloride to platinum. The method is as follows: Distil the solution containing formic acid with a small amount of phosphoric acid until the distillate is no longer acid. To the distillate add an excess of platinic chloride and sufficient acetic acid to make the solution strongly acid. Boil for 1 hour beneath a reflux condenser. Filter, wash, dry and weigh the reduced platinum. This weight multiplied by 0.472 gives the amount of formic acid present.

The official method adopted by the Association of Official Agricultural Chemists and published in September, 1920, is based on the one proposed by Fincke (*Z. Nahr. Genussm.*, 21, 1; 22, 88, and *Biochem. Z.*, 51, 253). The apparatus consists of a steam generator, a 300 c.c. flask in which the sample is placed, a 500 c.c. flask containing a suspension of barium carbonate, a condenser and a 1 litre graduated flask. The flask containing the sample is provided with a spray trap, and the tip of the tube leading into the suspension of carbonate consists of a bulb pierced by a number of small holes to break the vapour into small bubbles. For thin liquids, like fruit juices, 50 c.c. are used; for heavy liquids and semi-solids, like syrups and jams, 50 grm. diluted with 50 c.c. of water. The sample is placed in the 300 c.c. flask and 1 grm. of tartaric acid is added, the 500 c.c. flask having been previously charged with a suspension of 2 grm. of barium carbonate in 100 c.c. of water. If much acetic acid is present, sufficient barium carbonate must be used so that at least 1 grm. remains at the end of the operation. The contents of both flasks are now heated to boiling and steam admitted from the steam generator, the steam passing first through the sample in the first flask,

then through the boiling suspension of barium carbonate, after which it is condensed and measured in the graduated flask. The distillation is continued until 1 litre of distillate has been collected, maintaining the volume of the boiling liquids as nearly constant as possible by heating with small Bunsen flames and avoiding charring of the sample. After 1 litre of distillate has been collected, the apparatus is disconnected and the barium carbonate suspension filtered, washing it with a little hot water. The filtrate and washings should measure about 150 c.c.; if they are more they should be boiled down to that volume. 10 c.c. of sodium acetate solution (50 grm. in 100 c.c.), 2 c.c. of 10% hydrochloric acid, and 25 c.c. of mercuric chloride reagent (100 grm. of mercuric chloride and 150 grm. of sodium chloride per litre) are now added, thoroughly mixed and the container placed in a boiling water-bath or steam bath for 2 hours. The precipitate is then filtered on a tared Gooch crucible, thoroughly washed with cold water and finally with a little alcohol. It is then dried in a boiling water oven for 30 minutes, cooled and weighed. The weight of the formic acid present is calculated by multiplying the weight of the precipitate found by 0.0975. If the weight of mercurous chloride obtained exceeds 1.5 grm., the determination must be repeated, using more of the mercuric chloride reagent or less of the sample. A blank test must be run with each new lot of reagents employed in the reduction, using 150 c.c. of water, 1 c.c. of 10% barium chloride solution, 2 c.c. of 10% hydrochloric acid, 10 c.c. of the sodium acetate and 25 c.c. of the mercuric chloride reagent, heating the mixture in a boiling water-bath for 2 hours. The weight of mercurous chloride obtained in the blank test must be subtracted from that found in the regular estimation.

**Propionic Acid,  $\text{HC}_3\text{H}_5\text{O}_2$ .**—This substance is of little commercial importance, but its detection and separation from its homologues are occasionally necessary.

Propionic acid is contained in crude oil of amber, in sour coconut milk and in certain wines, especially when the fermentation has been pushed too far. It is also produced by the fermentation of glycerol and lactic acid, and by many synthetic methods. Propionic acid is also formed in the fermentation of the giant kelps of the California coast (*Macrocystis pyrifera*). The main product of this fermentation is acetic acid, but considerable quantities of propionic and, in

lessening amounts, butyric, valeric and caproic acids are obtained. It closely resembles acetic acid, but has an odour recalling at once those of acetic and butyric acids. It boils at  $140^{\circ}$  and has a sp. gr. of 0.996 at  $19^{\circ}$ .

The *propionates* closely resemble the acetates; they are all soluble in water.

The following method is described by Linnemann for the separation of propionic acid from its lower homologues: The free acids are evaporated to dryness with excess of litharge. The residue is then treated with cold water and the liquid filtered. Basic propionate of lead dissolves, whilst any acrylate remains insoluble, together with most of the acetate and formate. The solution is boiled and stirred quickly, when the propionate separates suddenly and almost completely as a crystalline precipitate, soluble in cold water, but which may be filtered at a boiling heat from the remaining acetate and formate. The propionic acid of fermentation is said not to exhibit this reaction.

**Butyric Acid**,  $\text{HC}_4\text{H}_7\text{O}_2$ .—Two modifications of this acid are known.

**Normal butyric acid**,  $\text{C}_3\text{H}_7\text{COOH}$ , occurs ready formed in various natural products, and is frequently produced by the decomposition of animal and vegetable matter. Butyric esters exist in butter and cod-liver oil and butyric acid can be produced by a special fermentation of sugar.

Normal butyric acid is a colourless mobile liquid, having a smell at once resembling acetic acid and rancid butter. It is soluble in water, alcohol and ether in all proportions, but is not soluble in a concentrated solution of calcium chloride or common salt; hence it may be separated from its aqueous solution by saturating the liquid with calcium chloride and agitating with ether. From the ethereal layer it may be recovered by spontaneous evaporation or, as a salt, by agitation with excess of solution of sodium hydroxide.

Phelps and Palmer (*J. Biol. Chem.*, 1917, **29**, 199) separate butyric acid in biological products from formic and acetic acid by treating their barium salts with quinine sulphate, dissolving the quinine butyrate in carbon tetrachloride and weighing as quinine butyrate, which is identified by its m. p. The solvent must be evaporated at a low temperature in a current of air and the salt dried over potassium hydroxide in a desiccator to prevent decomposition. They

give the following m. p. and solubilities of the quinine salts in carbon tetrachloride:

	M. P.	SOLUBILITY
Formate.....	110-113°	1:16,000
Acetate.....	124-126°	1:2000
Propionate.....	110.5-111°	1:450
Butyrate.....	77.5°	1:25

Propionic and formic acid may be similarly separated, but the difference in the solubilities of the butyrate and the propionate is not sufficient for a complete separation.

For other methods of approximately separating butyric from acetic and valeric acids see page 683.

**Isobutyric acid**,  $\text{CH}(\text{CH}_3)_2\text{COOH}$ , occurs in carob beans and is among the acids derived from castor oil. It closely resembles the normal acid in its general properties, but has a lower b. p. and sp. gr. Its smell is less offensive than that of the normal acid obtained by the decomposition of butter or by fermentation of sugar. It requires 3 parts of cold water for solution, and is easily oxidised to acetic acid and carbon dioxide when heated with chromic-acid mixture.

All *butyrates* are soluble in water. Lead butyrate is a heavy liquid, which solidifies when cooled.

*Copper butyrate* forms bluish-green monoclinic crystals, which are sparingly soluble in water. The formation of this salt may be employed to distinguish butyric from valeric acid. (*See U. S. Pharmacopæia, 9th Revision, under zinc valerate.*)

The *isobutyrate*s closely resemble the butyrates, except those containing calcium and silver. *Normal calcium butyrate* is very soluble in cold water, but separates as a crystalline precipitate on heating the strong solution to 70°. The *isobutyrate* is more soluble in hot water, and separates, on cooling, as a crystalline magma.

Ethyl butyrate can be formed by heating a butyrate with alcohol and strong sulphuric acid. It has a fragrant odour of pineapple, and boils at 120°.

Ethyl butyrate is produced when butter-fat is saponified by alcoholic solution of a strong alkali. The reaction is easily brought about by adding a small piece of butter (it is not necessary to render out the fat) to a strong solution of sodium hydroxide in alcohol, and heating the mass cautiously until it foams actively. The liquid

is then quickly poured into a comparatively large volume of cold water, when the characteristic odour of the ester is easily noticed. The equation of the reaction is unknown. The test is a convenient one for distinguishing butter from straight butter substitutes, but is, of course, of no value for mixtures containing appreciable amounts of butter-fat.

**Valeric Acid; Valerianic Acid;  $\text{HC}_5\text{H}_9\text{O}_2$ .**—Four forms of this are possible, derived from the four primary amyl alcohols.

**Propyl-acetic acid; Normal valeric acid.**—This is obtained by synthetic methods, also from calcium lactate by the action of some fission fungi and by the action of an enzyme contained in the tissues of *Ascarides* on carbohydrates. It has an odour recalling that of butyric acid. It boils at  $185^\circ$  and has a sp. gr. of 0.9415 at  $20^\circ$ .

**Methylethyl-acetic acid.**—This can be obtained from the oil of the fruit of the *Angelica archangelica* L., and probably exists in small amount in valerian root. It is optically active, having the value  $[\alpha]_D = 17.85^\circ$ . Some synthetic forms are inactive by racemism, but the ordinary form of active amyl alcohol gives the dextro-rotatory form of the acid. It boils at about  $172^\circ$ .

**Isopropylacetic acid; Isovaleric acid.**—This is the common form, ordinarily called valerianic acid. It occurs in valerian root. It is optically inactive, but when prepared from valerian root often has slight optical activity, due, it is thought, to a small amount of the active isomer. Esters of this occur in dolphin and porpoise oils, in sweat, and in various other products and secretions of animals. It exists in valerian root and many *Compositæ*. It is a colourless, oily liquid, with an odour resembling old cheese. Its taste is sharp and acid, and it blanches the tongue. It dissolves in about 30 parts of cold water, and is readily soluble in alcohol, ether, chloroform or strong acetic acid. It is almost wholly removed from its aqueous solution by saturating the liquid with common salt or calcium chloride.

This acid has a sp. gr. of 0.937 at  $15^\circ$ , and boils as  $175^\circ$ . It forms a hydrate of the composition  $\text{C}_5\text{H}_{10}\text{O}_2, \text{H}_2\text{O}$ , having a density of 0.950 and boiling at  $165^\circ$ , but it is gradually dehydrated by distillation, the weaker acid coming off first. On the other hand, on distilling the dilute aqueous acid, the first portions of the distillate are most strongly acid.

**Trimethylacetic acid** is solid at ordinary temperatures, melting at  $35.4^{\circ}$  to a liquid of 0.905 sp. gr. at  $50^{\circ}$ , and boiling at  $163.8^{\circ}$ . It is optically inactive.

**Reactions of Isovaleric Acid and Isovalerates.**—When isovaleric acid or an isovalerate is distilled with sulphuric acid and a little amylic alcohol, a fragrant ethereal liquid smelling of apples is obtained; this is amyl isovalerate.

Isovalerates are decomposed by acetic acid, with formation of isovaleric acid and an acetate; they are also decomposed by tartaric, citric and malic acids.

Isovalerates are mostly soluble in water. Iron and bismuth oxyisovalerates are insoluble. Silver and mercurous isovalerates are but slightly soluble, and aluminum isovalerate is insoluble. Neither this acid nor butyric gives a precipitate with an aqueous solution of zinc acetate. This fact distinguishes them from *caproic acid*, which throws down sparingly soluble zinc caproate as a white crystalline precipitate.

**Barium isovalerate** crystallises easily in triclinic scales or tables (in distinction from the same compound from active valeric acid), is soluble in 2 parts of cold water and sparingly soluble in alcohol. Barium *caprylate* requires 120 parts of cold water for solution, and is nearly insoluble in alcohol. Barium *caprate* is almost insoluble in water.

When concentrated isovaleric acid is agitated with solution of copper acetate, anhydrous copper isovalerate separates in oily drops which, in from 5 to 20 minutes, crystallise as greenish-blue monoclinic prisms or octohedra of hydrated copper isovalerate, moderately soluble in water and alcohol. The salt is less soluble in hot water than in cold, and hence the saturated solution becomes turbid when heated. This reaction distinguishes the acid from butyric acid, which forms with a moderately strong solution of copper acetate an *immediate* precipitate or turbidity of copper butyrate, of bluish-green colour, and crystallising in small monoclinic prisms. In using this test for analysis the acid must first be obtained free by distilling the salt with a moderate excess of sulphuric acid.

Isovaleric acid may be separated from most organic acids by converting it into the soluble lead salt. Acetic acid may be detected by neutralising any free acid with sodium hydroxide, and precipitating in the cold with excess of ferric chloride. In the presence of

acetic or formic acid, the filtered liquid will have a red colour. The insolubility of aluminum isovalerate might probably be employed for the separation of the acid from acetic or formic acid.

For other methods of estimating the acid and separating it from its homologues, see page 684.

**Commercial Valeric Acid and Valerates.**—The presence of *alcohol, acetic acid, butyric acid and valerates* in commercial valeric acid is indicated by the increased solubility of the sample, which should not be greater than 1 of the hydrated acid in 26 parts by weight of water. If the sample requires more than 30 parts of cold water for solution, the presence of *higher homologues*, or *valeral* (valeraldehyde,  $C_5H_{10}O$ ), is indicated. Acetic acid may be recognised as indicated on page 644. By neutralising the sample with an alkali, any amyl alcohol, valeric aldehyde or neutral ester will be left undissolved, as a turbidity or oily layer, and the amount may be estimated by measurement, or the mixture may be shaken with ether, and the ethereal liquid evaporated spontaneously. The solubility of valeric acid in a mixture of equal volumes of glacial acetic acid and water may be employed to separate it from valeral and esters, but not from amyl alcohol. The presence of butyric acid will be indicated by fractional distillation and by the composition of the salt obtained by saturating the acid with barium carbonate; also by the reaction with copper acetate.

Valeric acid should also be tested for non-volatile impurities, sulphuric acid, and hydrochloric acid. Valerates have been somewhat extensively used in medicine, especially the sodium, iron, zinc and bismuth salts. They are all more or less liable to adulteration, which in some instances is very gross. Thus, samples of zinc valerate are occasionally composed of the sulphate or acetate, and others have been met with which consisted of zinc butyrate impregnated with oil of valerian. Zinc valerate is also liable to adulteration with tartaric and citric acids, boric acid and other substances. Similarly, iron tartrate or citrate flavoured with valerian has been substituted for the iron valerate, and the quinine sulphate for the valerate. Ammonium valerate has been prepared by saturating calcium chloride with oil of valerian, and many similar frauds have been perpetrated.

Most of the above adulterations may be readily detected. The substitution of butyrate for valerate is best recognised by distilling

the salt with sulphuric acid diluted with an equal volume of water, and then applying the copper acetate and other tests to the distillate.

The most satisfactory ready test for valerates is to weigh or measure the layer of free acid which separates on decomposing the solid salt with sulphuric acid diluted with an equal volume of a saturated aqueous solution of zinc sulphate.

**Oxalic Acid.**—This acid is extensively formed in the physiological processes of plants and animals. It is usually converted into calcium oxalate, appearing as crystalline deposits (raphides) in cells of plants, but potassium hydrogen oxalate is sometimes found in plant juices. Calcium oxalate is often found in small amount in urine.

Oxalic acid is a product of the action of nitric acid, alkaline potassium permanganate and other oxidising agents on many organic substances.

On a large scale, the acid is usually made by the action of alkalis when starch, sawdust, straw, bran, or when similar vegetable matter is heated with potassium hydroxide an oxalate is formed. Wheat bran yields 150% of its weight of crystallised oxalic acid. Sodium hydroxide cannot be advantageously substituted, but with a mixture of the alkalis very satisfactory results are obtained. The product of the action is treated with water, and the solution treated with slaked lime. The alkalis are thus recovered. The calcium oxalate is separated and decomposed with sulphuric acid, the resulting acid being separated by evaporation and crystallisation.

Oxalic acid usually occurs crystallised with 2 molecules of water, in monoclinic prisms having a sp. gr. of 1.641 at 4°. Exposed to dry air, or in vacuo over oil of vitriol, the crystals lose water, become opaque, and form a white powder. The acid may be also obtained anhydrous by exposure to a gentle heat (60° to 70°). If at once heated to 100° the crystals melt, and it is then much more difficult to drive off the water. By dissolving ordinary oxalic acid in 12 parts of warm concentrated sulphuric acid, and allowing the solution to stand for several days, the anhydrous acid is deposited in transparent crystals, which on exposure to air absorb water and fall to powder.

Saturated solutions of oxalic acid lose acid at 100°, and the anhydrous acid may be readily sublimed. This furnishes a convenient

mode of obtaining the pure acid for analytical purposes. The acid should previously be rendered anhydrous by heating to  $60^{\circ}$  or  $70^{\circ}$ , and the temperature of the retort must be kept as constantly as possible at  $157^{\circ}$ . If allowed to rise to  $160^{\circ}$ , much loss of acid occurs, and an inferior product is obtained containing water and formic acid. The passage of a current of dry air greatly facilitates the sublimation.

Oxalic acid is colourless and odourless, and completely volatile by heat without charring.

100 parts of water dissolve 8 parts of crystallised oxalic acid at  $10^{\circ}$  and 345 parts at  $90^{\circ}$ . The solution is intensely sour, reddens litmus strongly, and is very poisonous. It decomposes carbonates, phosphates, chromates and many other salts, including fluorspar. Powdered oxalic acid completely decomposes sodium or calcium chloride when the mixture is heated. Prussian blue dissolves in oxalic acid to a clear blue liquid, sometimes employed as a blue ink. Solutions of oxalic acid are permanent in the dark, but when exposed to light the acid is rapidly decomposed.

Crystallised oxalic acid dissolves readily in cold and still more readily in boiling alcohol. It is but slightly soluble in ether, and is insoluble in chloroform, benzene or petroleum spirit.

Oxalic acid is not affected by boiling with moderately strong nitric or hydrochloric acid. Cold sulphuric acid has no action on it; but when heated with concentrated sulphuric acid, it decomposes into carbon monoxide, carbon dioxide and water.

When heated with glycerol, oxalic acid yields carbon dioxide and water at a moderate heat and formic acid at a higher temperature. This is the method commonly employed for producing formic acid.

Manganese and lead dioxides convert oxalic acid into carbon dioxide and water. Auric chloride and acid solutions of permanganates react similarly.

**Reactions of Oxalic Acid and Oxalates.**—An aqueous solution of oxalic acid presents the following analytical characters:

On addition of lime-water or solution of calcium acetate, a white precipitate of calcium oxalate is formed. The precipitate is insoluble in water, and not sensibly soluble in acetic or other organic acids. It is readily soluble in dilute mineral acids. It is decomposed by boiling with excess of sodium carbonate solution, with formation of insoluble calcium carbonate and soluble sodium oxalate. On gentle

ignition, calcium oxalate evolves carbon monoxide and leaves calcium carbonate. No blackening occurs. Solutions of soluble oxalates give the same reaction as oxalic acid with lime-water or calcium acetate, and react with calcium sulphate or chloride in addition. If previously neutralised by ammonium hydroxide, oxalic acid solutions are precipitated by the two latter reagents.

With solutions of barium, oxalic acid and oxalates react in a similar manner as with solutions of calcium, but the resultant barium oxalate is not so insoluble in water or acetic acid as the calcium salt.

On addition of dilute sulphuric acid and manganese dioxide, warm solutions of oxalic acid and oxalates produce effervescence, owing to the formation of carbon dioxide. The gas may be proved to be carbon dioxide by its reaction with lime-water.

In presence of dilute sulphuric acid, a warm solution of oxalic acid rapidly decolourises potassium permanganate. From strong solutions, the resultant carbon dioxide escapes with effervescence.

**Estimation of Oxalic Acid.**—Oxalic acid may be estimated with considerable accuracy by any of the following methods, the details of which may be found in most works on quantitative analysis:

(a) By precipitation as calcium oxalate. The solution should be hot and dilute, and mineral acids must be absent or previously neutralised by ammonium hydroxide. In the absence of other acids forming insoluble or nearly insoluble calcium salts (*e. g.*, sulphates, tartrates, citrates, phosphates), the solution may be exactly neutralised by ammonium hydroxide, and calcium chloride added. Any phosphate may be separated by digesting the precipitate with cold dilute acetic acid. In presence of sulphates, calcium sulphate should be employed as a precipitant. It is frequently preferable to have the solution acid with acetic acid or to precipitate the acid solution with calcium acetate, so as to avoid the precipitation of other calcium salts. Almost all calcium salts are soluble in acetic acid, except the oxalate, racemate, and fluoride. Racemates may be previously removed by precipitation with potassium acetate in presence of alcohol. The separation of oxalates and fluorides is rarely required in practice, but, if required the oxalate can be determined by titrating the precipitate with standard potassium permanganate. The precipitate of calcium oxalate, however produced, is to be well washed and then treated in one of the following ways:

1. It is dried at  $100^{\circ}$ , and weighed as calcium oxalate.
2. It is ignited, moistened with ammonium carbonate, again gently ignited, and weighed as calcium carbonate.
3. It is moistened on the filter with strong sulphuric acid, and the whole ignited again, moistened with sulphuric acid, re-ignited, and finally weighed as calcium sulphate.
4. It is ignited thoroughly, and the resultant calcium oxide and carbonate titrated with standard acid.
5. The filter is placed in a beaker together with water and dilute sulphuric acid, and the liquid is titrated with standard potassium permanganate.

Of these methods, the last two are perhaps the best, because they are the least affected by impurity in the precipitate. Process 5 aims at the direct estimation of the oxalate, and may be applied to a precipitate containing phosphate, carbonate, or sulphate; but tartrate, racemate, and most organic salts must be absent from the precipitate.

(b) By treatment with dilute sulphuric acid and manganese dioxide in a carbon dioxide apparatus. This process is conducted precisely as in the valuation of a manganese ore, except that excess of the manganese dioxide is used instead of excess of the oxalate. 44 parts by weight of carbon dioxide lost by the apparatus represent 63 of crystallised, or 45 of anhydrous oxalic acid.

(c) By titration with standard permanganate. The solution of the oxalate must be free from other readily oxidisable substances, and should be warm, dilute, and pretty strongly acidified with sulphuric acid. The permanganate is added gradually, with constant stirring, until the liquid acquires a permanent pink tint. The permanganate is preferably standardised with pure oxalic acid. N/10 potassium permanganate is a suitable strength. Each cubic centimeter of this solution will oxidise 0.0063 grm. of crystallised or 0.0045 grm. of anhydrous oxalic acid. The process can be employed for titrating a precipitate of calcium oxalate.

In cases of poisoning by free oxalic acid, the acid extracted from the stomach and intestines is chiefly uncombined, but that obtained from the liver, kidneys, heart and urine is in combination.

J. M. Albahary (*J. Soc. Chem. Ind.*, 1909, 28, 738) proposes a new method of estimating *oxalic acid* in cocoa and chocolate which gives more satisfactory results than other existing processes and is also

applicable in the analysis of plant juices. It is based on the fact that magnesium salts precipitate phosphates and purine bases in alkaline solution whilst oxalates remain dissolved as magnesium alkali oxalates.

The procedure is as follows:

50 grm. of the sample are dried over sulphuric acid to constant weight and then heated on a water-bath for 1 hour with 50 c.c. of 10% sodium carbonate solution, the volume being kept constant during the heating by adding water from time to time. The mixture is then treated with 50 c.c. of a solution containing 10% of magnesium chloride and 20% of ammonium chloride, a quantity of animal charcoal being added to retain mucilaginous substances. After concentrating by heating for 1 hour, with occasional shaking, the mixture is filtered hot by means of suction, and the residue washed with boiling water. The filtrate is concentrated, made strongly alkaline with ammonia and, after standing 12 hours, is filtered. The filtrate is treated with a slight excess of calcium chloride and made faintly acid with acetic acid. After standing 12 hours the calcium oxalate is filtered off and the oxalic acid determined in the usual way. This gives total oxalates. If the preliminary treatment with sodium carbonate be omitted, the final result will give soluble oxalates and the difference between the two results gives the amount of calcium oxalate present.

**Commercial oxalic acid** is not very liable to intentional adulteration.

*Organic matters* other than oxalic acid are recognised by the charring or darkening of the sample when heated, or on warming with concentrated sulphuric acid.

*Fixed mineral impurities* are left as a residue on igniting the sample in the air. If the ignited residue effervesces on addition of dilute acid, an *acid oxalate* is probably present in the sample. Sensible quantities of *lead* and other heavy metals are sometimes met with. *Sulphuric acid* and *acid sulphates* are sometimes present in considerable amount. The solution of such samples gives a white precipitate with barium chloride. The same impurities occur in commercial ammonium oxalate.

**Oxalates.**—These salts require but little special description. The metals of the potassium group form 3 classes of oxalates, the potassium salts having the formulæ  $K_2C_2O_4 \cdot H_2O$ ;  $KHC_2O_4 \cdot H_2O$ ; and

$\text{KH}_3(\text{C}_2\text{O}_4)_2 \cdot 2\text{H}_2\text{O}$ . The acid salts are the least soluble. The oxalates of most other metals are insoluble, or nearly insoluble, in water. This is true of the oxalates of barium, strontium, calcium, copper, magnesium, manganese, cobalt, nickel, zinc, lead and silver. The first 4 of these retain 1 molecule of water on drying at  $100^\circ$ . The remainder retain 2 molecules, with the exception of the lead and silver salts, which are anhydrous. Ferrous oxalate is but sparingly soluble, but ferric oxalate is readily so, at least in presence of free oxalic acid; hence the use of oxalic acid for removing ink stains and dissolving Prussian blue. All the insoluble oxalates are soluble in dilute nitric acid, but they are generally insoluble in acetic acid. The estimation of the oxalic acid may be readily effected by the methods described on page 699.

On ignition, oxalates containing metals not easily reducible evolve carbon monoxide, and leave carbonates. These may sometimes be further decomposed if the temperature be excessive. Oxalates containing more easily reducible metals, when heated to redness in a closed vessel, usually leave the metal and evolve carbon dioxide. This reaction occurs even at  $100^\circ$  in the case of gold; hence gold is reduced from its solutions by boiling with an oxalate.

Pure oxalates do not char on ignition.

**Succinic Acid.**—Succinic acid occurs naturally in amber and in certain lignites; it is produced during the alcoholic fermentation of sugar; and by the fermentation of malic acid and many other substances, especially under the influence of putrefying casein; also by the action of nitric acid on the fatty acids and fats, and it exists ready formed in several plants.

It may be obtained by the dry distillation of amber, the aqueous distillate being filtered while hot to separate oil, when crystals of the acid are deposited on cooling, and may be purified by boiling with nitric acid, followed by recrystallisation from water.

Succinic acid bears the same relation to butylene (tetrene) alcohol that oxalic acid does to ethylene glycol, and may be produced from butylene alcohol by oxidation. It may also be obtained by the deoxidation of tartaric or malic acid, which contain, respectively, 2 and 1 atom more of oxygen than does succinic acid.

Succinic acid crystallises in colourless, oblique rhombic prisms or plates. When heated to  $130^\circ$ , it emits suffocating fumes, and at  $180^\circ$  melts. When the heat is increased to  $235^\circ$  the acid boils and

sublimes as succinic anhydride, which melts at  $120^{\circ}$ . When heated strongly in the air, succinic acid burns with a blue smokeless flame.

Succinic acid is soluble in about 18 parts of cold and 0.8 boiling water. It dissolves readily in alcohol and sparingly in ether, but is insoluble in chloroform, benzene, petroleum spirit, turpentine or carbon disulphide. Nitric acid, chlorine and chromic acid have no action on succinic acid, and it is soluble without change in strong sulphuric acid. Permanganates have no action on a cold acid solution, but when heated in presence of free alkali produce oxalic acid.

**Reactions of Succinic Acid.**—In its analytical characteristics succinic acid somewhat resembles benzoic acid, but differs from it in not being precipitated from a strong solution of its salts by hydrochloric acid; in being precipitated by ammoniacal solution of barium chloride even from a dilute solution; and by being insoluble in chloroform, and therefore not removable from an acid solution by agitation with that liquid. Magnesium benzoate is soluble in alcohol, but the succinate is insoluble.

Ferric chloride, if first treated with as much ammonium hydroxide as it will bear without precipitation, will throw down from neutral solutions of soluble succinates a bulky cinnamon-brown basic ferric succinate, some free succinic acid being simultaneously produced, and the solution acquiring an acid reaction. Benzoates, under similar circumstances, give a flesh-coloured precipitate, and cinnamates a yellow. The precipitate may be filtered off, washed and decomposed by boiling with excess of dilute ammonium hydroxide. The filtered liquid, if mixed with barium chloride and an equal volume of alcohol, gives a white precipitate of barium succinate. By the above combination of reactions, succinic acid may be readily identified and separated from other organic acids. The process might possibly be made quantitative. For such a purpose, sodium acetate should be added to the liquid containing the iron precipitate, and the whole boiled, the precipitate produced being first boiled and then washed with dilute ammonium hydroxide, the liquid being then concentrated and precipitated by alcohol and barium chloride. Neutral succinates containing alkali metals may also be precipitated pretty completely by adding barium chloride to the boiling solution.

*Commercial succinic acid* has usually more or less of a brown colour, and somewhat of the odour of empyreumatic oil of amber, which impurity may be removed by agitation with petroleum spirit.

A *facticeous succinic acid* has been prepared by adding a little oil of amber to tartaric acid, ammonium chloride or potassium hydrogen sulphate.

*Inorganic impurities* and adulterants will be left on igniting the substance. *Cream of tartar* leaves potassium carbonate on ignition; it has been found in succinic acid to the extent of 50%. Barium sulphate may be recognised by its insolubility and other characters; and boric acid by the reddish-brown colour imparted to turmeric paper when the ash is acidulated with hydrochloric acid and the solution evaporated in contact with it. Heavy metals may be recognised by the usual tests.

*Foreign organic acids* may be detected by their special reactions. Thus *oxalic acid* will be precipitated on adding calcium acetate (or a mixture of calcium chloride and ammonium acetate) to the aqueous solution of the sample; *tartaric acid* by potassium acetate and alcohol; *citric acid* by the precipitate formed on adding excess of lime-water and boiling. *Benzoic acid* may be detected by its solubility in carbon disulphide or warm petroleum spirit, and by its separation on treating the precipitate produced in the neutralised liquid by ferric chloride with hydrochloric acid.

*Ammonium chloride* may be recognised by the tests for ammonium salts and chlorides.

*Sugar* and various other impurities cause charring on warming the substance with sulphuric acid.

A useful method of examining succinic acid is to dissolve 1 grm. of the sample in 15 c.c. of hot alcohol, in which it should be completely soluble. When cold, one-half the solution is mixed with an equal volume of chloroform, and the other with an equal volume of ammonia. Complete mixture should occur in both cases. If the result of the test is satisfactory, and the sample leaves no sensible quantity of ash, and does not notably darken with strong sulphuric acid, the substance may be considered pure.

After a critical study of all the existing methods for the estimation of *succinic acid in wine*, C. von der Heide and H. Steiner (*Zeit. Unters. Nahr. Genussm.*, 1909, **17**, 291; *Zeit. anal. Chem.*, 1912, **51**, 628) recommend that the following method be used when accurate results are desired:

Fifty c.c. of wine are evaporated in a 200 c.c. casserole to remove all alcohol. After the addition of 1 c.c. of 10% barium chloride solu-

tion and phenolphthalein, the solution is neutralised with powdered barium hydroxide, the excess of barium is removed by means of carbon dioxide and then 85 c.c. of 95% alcohol are added to the mixture whilst constantly stirring. After 2 hours the precipitate of barium succinate, tartrate, malate and other barium salts is collected on a filter, washed with a small quantity of 80% alcohol and then washed back into the casserole with hot water. The solution is concentrated to remove all alcohol, 5% potassium permanganate solution is then added in 3 c.c. portions until the red colour persists for 5 minutes. An additional 5 c.c. of permanganate is added and the mixture heated on a water-bath for 15 minutes. The excess of permanganate is reduced with sulphurous acid and, after acidification with sulphuric acid, more sulphurous acid is added until the manganese dioxide is dissolved. The mixture is then evaporated to about 30 c.c. and after acidification with sulphuric acid until about 10% of free acid is present, is extracted with ether for 12 hours in a percolating apparatus. The ethereal solution is evaporated with water and the residual aqueous solution is made alkaline with barium hydroxide. The mixture is then heated on a water-bath for 10 minutes, excess of barium is precipitated as carbonate, and the solution cooled and filtered. 20 c.c. of N/10 silver nitrate are added to the solution in a 100 c.c. flask and diluted to the mark. After standing 2 hours the excess of silver in a 50 c.c. portion is titrated by the Volhard method.

**Malic Acid.**—Malic acid is contained in apples, pears and many fruits used for domestic purposes. It is usually prepared from rhubarb stalks or mountain-ash berries.

Malic acid crystallises in groups of 4 or 6 sided prisms, which are colourless and odourless, and readily fusible. Malic acid is deliquescent and readily soluble in water, alcohol and ether. The aqueous solution has an agreeable acid taste, and becomes mouldy on keeping. In contact with ferments, especially putrid cheese, the solution of malic acid yields succinic and acetic acids and sometimes butyric acid.

When heated in a small retort to about 180°, free malic acid melts and evolves vapours of maleic and fumaric acids, which crystallise on the cooler parts of the retort and receiver. Fumaric acid forms slowly at 150°, and mostly crystallises in the retort, in broad, colour-

less, rhombic or hexagonal prisms, which vaporise without melting at about  $200^{\circ}$ , and are soluble in 250 parts of cold water, and easily in alcohol and ether. Maleic acid is the chief product if the temperature be suddenly raised to  $200^{\circ}$ . This substance crystallises in oblique rhomboidal prisms, which melt at  $130^{\circ}$ , vaporise at about  $160^{\circ}$ , and are readily soluble in water and alcohol. The behaviour of malic acid on heating is of value owing to the few characteristic tests for this acid. Maleic and fumaric acids are stereo-isomers.

Malic acid exhibits optical activity. It exists in two forms: dextrorotatory and lævorotatory.

By the action of hydriodic acid, under pressure, malic acid is converted into succinic acid. Nitric acid and alkaline solutions of permanganate oxidise malic acid. Concentrated sulphuric acid darkens malic acid and malates very slowly on warming. When boiled with dilute sulphuric acid and potassium dichromate, malic acid evolves an odour of ripe fruit.

No malate is quite insoluble in water; only a few are soluble in alcohol. A solution of calcium chloride does not precipitate malic acid or malates in the cold (distinction from oxalic and tartaric acids); only in neutral and very concentrated solutions is a precipitate formed on boiling. (Citrates are precipitated from neutral boiling solutions by calcium chloride, unless the liquid is very dilute.) The addition of alcohol after calcium chloride produces a bulky, white precipitate of calcium malate, even in dilute neutral solutions. Thus, if the liquid be filtered first cold (to remove oxalic and tartaric acids), and then boiling hot (to remove citric acid), the malic acid can be precipitated on addition of 2 volumes of alcohol. This precipitate may contain calcium sulphate or succinate, but will be free from formate, acetate, or benzoate except, that if more than 2 volumes of alcohol are added, calcium formate precipitates. On boiling the precipitate with a moderate quantity of water, the malate will be dissolved, and tannate and sulphate left almost wholly behind. The precipitate produced by calcium chloride and alcohol may also be tested for malic acid (after drying it to get rid of all trace of alcohol) by decomposing it with dilute sulphuric acid, and boiling the filtered liquid with a *small* quantity of potassium dichromate. If the liquid remains yellow, succinic acid alone is likely to be present; but if green and without odour, citric acid is probably present either with or without succinic acid. If the liquid becomes green and

evolves an odour of ripe fruit, malic acid is present, and possibly either or both succinic and citric acid, in addition.

Solutions of lead acetate precipitate malates (more perfectly after neutralisation with ammonia) as a white (and frequently crystalline) precipitate of lead malate, which, on boiling for a few minutes, sets under the liquid to a transparent, waxy, semi-solid. This characteristic reaction is obscured by the presence of other organic acids. The precipitate is very sparingly soluble in cold water; somewhat soluble in hot water. Lead malate is soluble in strong ammonia, but is not readily dissolved by a slight excess. (Distinction from tartrate and citrate.) It dissolves in ammonium acetate solution, and on mixing the liquid with 2 volumes of alcohol is reprecipitated. (Lead succinate remains in solution.)

The precipitate of lead malate may be washed with a mixture of 2 volumes of alcohol and 1 of water.

If the precipitate of lead malate is treated with excess of ammonium hydroxide, dried on the water-bath, moistened and triturated with alcoholic ammonia, and then treated with absolute alcohol, only ammonium malate dissolves; ammonium citrate, tartrate, and oxalate being insoluble in absolute alcohol. Malic acid may be separated from other organic acids in solution by adding ammonium hydroxide in slight excess, and then 8 or 9 volumes of strong alcohol, which precipitates all but the ammonium malate. The method may be conveniently applied to the solution of the acids obtained by suspending the lead salts in water and passing hydrogen sulphide through the liquid.

If the alcoholic solution of ammonium malate is precipitated by lead acetate, and the lead malate obtained filtered off, washed with alcohol, dried at  $100^{\circ}$  and weighed, the weight obtained, multiplied by 0.3953, gives the quantity of malic acid present.

**Estimation of Malic Acid.**—A method of estimating malic acid in fruit juices is described by P. B. Dunbar and R. F. Bacon (*U. S. Dept. Agr., Bur. Chem., Circular 76*; *J. Ind. Eng. Chem.*, 1911, **3**, 826). This method has been supplemented by D. S. Pratt (*U. S. Dept. Agr., Bur. Chem., Circular 87*) with a preliminary treatment which removes several mechanical difficulties in the original process. A further modification of this method, as tentatively adopted and published by the Association of Official Agricultural Chemists in September, 1920, is carried out as follows:

I. *For fruit juices* and similar products containing no tartaric acid and not over 15% of sugars and in which the colour does not interfere with polarisation: filter the sample, if necessary to secure a solution which can be readily polarised, and polarise with white light, using a 200 mm. tube, if possible. A 100 or a 50 mm. tube may be used, but all readings must be calculated on the basis of a 200 mm. tube.

If the sample contains free mineral acid, transfer a measured portion (75 c.c.) to a 100 c.c. graduated flask, add enough standard alkali, calculated from the total acidity determination, to neutralise, dilute to the mark, mix well and filter. If no free mineral acid is present, it is unnecessary to neutralise. If neutralised, proper correction must be made for dilution.

Transfer 25 c.c. of the sample or the neutralised solution to a flask graduated at 25 and 27.5 c.c., add about 2.5 gm. of powdered uranyl acetate and shake vigorously at intervals for 3 hours, keeping the mixture well protected from light. If all the uranyl acetate dissolves, add more so that a small amount remains undissolved at the end of 3 hours. Dilute the solution to the 27.5 c.c. mark with saturated uranyl acetate solution, mix well and filter, if necessary, through a folded filter. Polarise, if possible in a 200 mm. tube. Multiply the reading by 1.1 to correct for the dilution.

Multiply the difference between the two readings, in degrees Ventzke calculated to the basis of a 200 mm. tube, by the factor 0.036 to obtain the weight of malic acid in the sample in gm. per 100 c.c.

Make all polarisations at the same room temperature with white light. The average of six readings should be taken. In the case of dark colored fruit juices which cannot be polarised readily, approximately quantitative results may be obtained by adding to the solutions a few drops of bromine, shaking thoroughly and filtering just before polarisation.

II. *Approximate estimation* for fruit juices and similar products containing no tartaric acid and more than 15% of sugars (see also J., A.O.A.C. 1915, 1, 480): weigh out 25 gm. of the sample and transfer to a 600 c.c. beaker with a little 95% alcohol (by volume). Add alcohol a little at a time until 200 c.c. have been added, stirring the mixture well, and warming, if necessary, to insure solution of all alcohol-soluble substances. Filter on a Buchner funnel, using

suction, and thoroughly wash the precipitated pectins and insoluble matter with 95% alcohol, disregarding the slight turbidity which may appear in the filtrate after the washings have been added. From the total acidity determination calculate the amount of N/4 barium hydroxide required nearly to neutralise the acidity in the 25 grm. of sample taken. Add this amount to the combined filtrate and washings in an Erlenmeyer flask, stir until reaction is complete and add 3 to 5 drops, or more if required, of 50% barium acetate solution to insure an excess of barium. Make up the volume of the mixture to about 375 c.c. (not less) with alcohol, and boil under a reflux condenser until the precipitate settles readily after shaking. This may require 3 to 4 hours. Filter with suction and thoroughly wash the precipitate in the flask and on the filter with alcohol. Transfer the portion on the filter to the flask, rinsing the paper with a jet of hot water. Digest the precipitate with hot water, containing 2 grm. of sodium sulphate in solution, until the reaction is complete, and boil until the barium sulphate precipitate settles readily. Concentrate by evaporation, if necessary, and transfer to a 100 c.c. volumetric flask with a little hot water, cool, make up to volume with water and filter. Polarise a portion of this filtrate. Transfer 25 c.c. of the filtrate to a flask graduated at 25 and 27.5 c.c., add uranyl acetate and polarise, proceeding exactly as in the method given above.

III. *Approximate estimation* for products containing tartaric acid (see also *J.*, *A.O.A.C.* 1915, 1, 480, and *Bur. Chem. Bull.* 162, 56): Prepare the sample as directed above up to the point of filtrating and washing the precipitate of barium malate, then dry the precipitate thoroughly and transfer the portion on the filter to the flask, rinsing the paper with a jet of hot water. Digest the precipitate with hot water, transfer to a 100 c.c. volumetric flask with a little hot water, cool, make up to volume with water and filter to remove insoluble barium tartrate. This amount of water is sufficient to dissolve barium malate up to amounts of about 0.9 grm. If larger amounts are present, more than 100 c.c. of water must be used. The amount of barium tartrate dissolved by hot water is so small as to affect only slightly the polarisation after the treatment with uranyl acetate. From here on the procedure is exactly as outlined above.

To estimate malic acid in cane and maple products the method of P. A. Yoder (*J. Ind. Eng. Chem.*, 1911, 3, 563) should be used.

A colour test to distinguish between *succinic* and *malic acids* is given by W. O. de Coninck (*Bull. Soc. Chem.*, 1914, 15, 93) as follows:

Heat a concentrated aqueous solution of the acid with calcium salicylate. Succinic acid gives a permanent rose colour which does not fade on standing in sunlight for several days. Malic acid gives a similar colour, but on 15 to 20 minutes' boiling it darkens and then disappears, leaving a yellowish liquid.

**Tartaric Acid.**—Tartaric acid occurs in some plant juices. Grape juice is the only important source. The deposit formed on the sides and bottom of the vessels in which wine is manufactured consists largely of calcium and potassium tartrates. After purification, it is treated with calcium carbonate and calcium sulphate, by which a nearly insoluble calcium tartrate is produced, and this, when decomposed with sulphuric acid, yields free tartaric acid, which is obtained in crystals by cooling the concentrated liquid.

Three distinct forms of tartaric acid exist. Their chief physical and chemical differences are as follows:

**Dextrotartaric, ordinary tartaric acid**, forms anhydrous, hemihedral, monoclinic crystals, the aqueous solution of which turns the plane of polarisation to the right, the value for  $[\alpha]_D$  at 16° being 13.1° for a 15%, and 14.7° for a 2% solution. The crystals fuse at 135°, have a sp. gr. of 1.74 to 1.76, and are readily soluble in absolute and dilute alcohol.

In the following article this acid and its salts are always referred to unless otherwise stated.

**Lævotartaric acid** forms anhydrous crystals, the aqueous solution of which turns the plane of polarisation of a luminous ray to the left, the rotation being equal and opposite to that produced by dextrotartaric acid.

**Inactive, or mesotartaric acid**, is produced by prolonged heating of dextrotartaric acid to 165° with a small proportion of water. It is optically inactive, but, unlike racemic acid, is not resolvable into two acids. Mesotartaric acid is very soluble in water, forms crystals containing 1 molecule of water, and yields calcium and potassium

hydrogen salts more soluble than the corresponding salts of ordinary tartaric acid.

**Racemic acid**, often described as a fourth form of tartaric acid, is really an association of equal quantities of the active forms and is optically inactive. It can be separated into the two forms and can also be obtained by mixing equal amounts of them. It occurs with ordinary tartaric acid in crude tartars. It forms crystals containing 1 molecule of water, which effloresce in the air, and become completely anhydrous at  $100^{\circ}$ ; the resultant anhydrous acid melts at about  $200^{\circ}$ . Racemic acid is soluble in 5 parts of cold water, and with difficulty in cold alcohol. The calcium racemate is less soluble in water than calcium dextrotartrate, and is also distinguished by its insolubility in acetic acid and in ammonium chloride solution.

The slighter solubility of calcium racemate as compared with calcium dextrotartrate has led to the suggestion of a method for detecting the latter by adding a solution of levotartaric acid to the liquid to be tested, then calcium chloride and neutralising the solution. The levotartaric acid will associate with an equal portion of dextrotartaric acid, if any is present, and the highly insoluble calcium salt will precipitate.

Ordinary tartaric acid is soluble in 0.7 part of cold and 0.5 part of boiling water; in 1.6 parts of cold alcohol (95%) and in about 0.2 part of boiling alcohol; in 250 parts of ether and is nearly insoluble in chloroform, benzene and petroleum spirit.

The following table by H. Schiff shows the sp. gr. of aqueous solutions of tartaric acid:

PERCENTAGE BY WEIGHT OF TARTARIC ACID	SP. GR. AT $15^{\circ}$
33.0	1.1654
22.0	1.1062
14.67	1.0690
11.0	1.0511
7.33	1.0337
3.67	1.0167

Unsterilised aqueous solutions of tartaric acid (especially when dilute) gradually decompose on account of the growth of mould. The change may be prevented by the addition of a little phenol. Many tartrates decompose when kept in a moist state.

Most oxidising agents convert tartaric into formic acid. Ammonio-silver nitrate is reduced with formation of carbonic and oxalic acids. In dilute solution, tartaric acid reduces gold and platinum chlorides, and converts mercuric chloride into calomel.

**Detection and Estimation of Tartaric Acid and Tartrates.**—Tartaric acid and tartrates are charred when heated with concentrated sulphuric acid of 1.845 sp. gr. The reaction may be used to distinguish a tartrate from a citrate or to detect tartaric acid in presence of citric acid. For this purpose, 1 grm. of the sample should be treated with 10 c.c. of pure concentrated sulphuric acid (free from nitrous compounds), and the mixture heated to 100° for 40 minutes. Citric acid gives only a yellow colour when thus treated, but if 1% of tartaric acid be present, the liquid has a distinct brown shade, and this becomes still more marked with larger proportions.

If a drop of ferrous sulphate solution is added to a solution of tartaric acid or a soluble tartrate, then a few drops of hydrogen peroxide, and the mixture finally treated with excess of sodium hydroxide, a fine violet is produced, which in strong solutions is so deep as to appear almost black. The colour is discharged by sulphurous acid. If potassium ferrocyanide is added to the violet liquid, and then sufficient dilute sulphuric acid to acidify the solution, the iron may be filtered off and a colourless filtrate obtained which again gives the violet colour on addition of a ferrous salt. The colourless filtrate reduces silver and mercury compounds, potassium dichromate and permanganates. After addition of excess of alkali it precipitates cuprous oxide from Fehling's solution in the cold; on heating, metallic copper is separated.

Acid solution of a permanganate or sodium hypochlorite may be substituted for the hydrogen peroxide in the foregoing test, if care be taken to avoid excess, but the result is not so satisfactory. Heavy metals and oxidising agents must be absent. Citric, malic, succinic, oxalic and acetic acids and sugar were found by H. J. H. Fenton, the observer of the reaction, to give no similar coloration (*Chem. News*, 1876, **33**, 190; 1881, **43**, 110).

Soluble tartrates in neutral solution give white calcium tartrate on addition of calcium chloride. The precipitate is nearly insoluble in cold water; soluble in solutions of many ammonium salts; soluble (after washing) in a cold solution of sodium hydroxide, but reprecipitated on boiling; soluble in acids (including acetic); and con-

verted by heating with a neutral solution of copper chloride into insoluble copper tartrate. Calcium citrate yields soluble copper citrate. Calcium tartrate may also be conveniently examined by dissolving it in the smallest possible quantity of acetic acid, adding excess of potassium chloride solution and stirring vigorously, when the potassium hydrogen tartrate will be thrown down.

The reducing action of tartaric acid on silver compounds is a delicate test, but is liable to failure if certain conditions are not observed. The solution of tartaric acid, or alkali-metal tartrate (all other metals being first removed), is rendered acid with nitric acid, *excess* of silver nitrate added, and any precipitate filtered off. To the solution, *very dilute* ammonium hydroxide is added until the precipitate at first formed is nearly redissolved. The solution is again filtered, and the filtrate heated nearly to boiling for a few minutes, when a brilliant mirror will be formed on the sides of the tube. Citric acid does not reduce silver under similar circumstances, but gives a precipitate on continued boiling.

Tartaric acid prevents the precipitation of many metallic solutions by alkalis, stable double tartrates being formed. For the separation of heavy metals from tartrates, hydrogen sulphide or sodium sulphide must be employed, according to the metals present. The filtrate may be concentrated, and any barium, strontium, calcium or magnesium present thrown down by boiling with sodium carbonate. Aluminum is not separated by either of the above precipitants, but the tartaric acid can be detected and estimated in the solution without removing it.

The best method of direct estimation of tartaric acid is to precipitate it in the form of potassium hydrogen tartrate. When the free acid is to be estimated, either alone or mixed only with citric acid, the method described under citric acid should be employed. For the estimation of tartaric acid in tartrates and in the various natural and artificial products of tartaric acid manufactories, processes are given below.

Like the corresponding salts of other organic acids, tartrates containing metals not easily reducible leave on gentle ignition a residue of carbonate or oxide, and by dissolving this residue in standard acid and ascertaining the amount of acid neutralised by titrating the excess with standard alkali, an accurate estimation can

be effected, and if it is known whether the tartrate was originally acid or neutral, an estimation of the acid itself is obtained.

Tartaric acid and hydrogen tartrates neutralise alkalis completely.

The tartaric acid in *tartrates containing organic bases* may generally be ascertained by precipitation as potassium hydrogen tartrate.

The alkyl tartrates are unimportant. Ethyl tartrate may be decomposed by heating with alcoholic sodium hydroxide and potassium hydrogen tartrate precipitated by adding excess of acetic acid.

**Tartaric acid in wine** may exist in the free state, and as calcium and potassium hydrogen tartrates, and ethyl tartrate is probably often present (see page 235).

The Association of Official Agricultural Chemists has adopted the following methods for the estimation of total and free tartaric acid and cream of tartar in wines and similar products:

*Total Tartaric Acid* (Official) (*U. S. Bur. Chem. Bull.*, 162, 72).—Neutralise 100 c.c. of the wine with N/1 sodium hydroxide, calculating the number of cubic centimeters alkali necessary from the total acidity. If the volume of the solution be increased more than 10% by this addition, evaporate to about 100 c.c. Add to the neutralised solution 0.075 gm. of tartaric acid for each cubic centimeter of N/1 alkali added and, after the tartaric acid has dissolved, add 2 c.c. of glacial acetic acid and 15 gm. of potassium chloride. After this has dissolved, add 15 c.c. of 95% (by volume) alcohol, stir vigorously, until the potassium hydrogen tartrate begins to precipitate, and then let stand in an ice box for at least 15 hours. Decant the liquid from the separated potassium hydrogen tartrate on a Gooch crucible prepared with a very thin film of asbestos or on a filter paper in a Buchner funnel. Wash the precipitate and filter 3 times with a few cubic centimeters of a mixture of 15 gm. of potassium chloride, 20 c.c. of alcohol and 100 c.c. of water, using not more than 20 c.c. of the wash solution in all. Transfer the asbestos or paper and precipitate to the beaker in which the precipitation was made, wash out the Gooch crucible or Buchner funnel with hot water, using about 50 c.c. in all, heat to boiling and titrate the hot solution with N/10 sodium hydroxide, using phenolphthalein as an indicator. Increase the number of cubic centimeters of N/10 alkali required by 1.5 c.c. to allow for the solubility of the precipitate. One c.c. of N/10 is equivalent, under these conditions, to



The limit of arsenic adopted is 1/100 gr. per pound (1.4 parts  $\text{As}_2\text{O}_3$  per million, or 0.00014%). There is no difficulty in obtaining tartaric acid in which arsenic falls considerably below this figure (Cf. A. W. McFadden, Local Government Board Report, Inspector of Foods, No. 2, 1907).

**Lead.**—The limit of lead generally adopted in commerce in Great Britain is 20 parts per million (0.002%), as recommended by McFadden (Cf. Tatlock and Thomson, *Analyst*, 1908, **33**, 173; T. F. Harvey and J. M. Wilkie, *Chem. and Drug.*, 1909, **75**, 92). Many methods have been suggested for making the test, of which the following are probably the most reliable:

*Method I.*—Based on C. A. Hill's method (*Chem. and Drug.*, 1905, p. 388) which is, like the following one, a modification of Warrington's original colorimetric method (*J. Soc. Chem. Ind.*, 1893, **12**, 97). Hill's process has been adopted by the 1914 British Pharmacopœia, and is described therein in detail.

A standard lead solution is prepared containing 5 parts of lead per million, by dissolving pure metallic lead in a minimum quantity of nitric acid (equal parts of concentrated acid and water) and suitably diluting; it is best to prepare a standard stock solution of lead, containing say 1 grm. of lead per litre, and then, when required, to dilute this for use to the above concentration. The standard solution may also be prepared from pure lead nitrate or from crystalline lead acetate.

For the test, 7 grm. of the sample are weighed out, and a separate portion of 2 grm. for the colorimetric comparison. Each portion is dissolved in about 10 to 15 c.c. of water, and to the 2 grm. portion are added as many cubic centimeters of the standard lead solution as there are suspected to be parts per million of lead in the tartaric acid tested. Thus to compare with 15 parts of lead per million, 15 c.c. of the standard lead solution are used. To each solution are then added 1 to 2 c.c. of 10% potassium cyanide solution and 13 c.c. of ammonia of sp. gr. 0.880, and the solutions are boiled for half a minute or longer so as to get both colourless if possible. They are next poured into two 50 c.c. Nessler cylinders of clear white glass and diluted to an equal volume (50 c.c.), any difference of colour being corrected, if necessary, by the addition of a drop or two of a very dilute solution of caramel. To each solution are then added 1 to 2 drops of a freshly prepared colourless ammonium sulphide

solution (obtained by saturating ammonia (sp. gr. 0.880), diluted with an equal volume of water, with hydrogen sulphide gas, carefully washed by passing through water). The colour of the two solutions is compared by examining them, placed on a sheet of white paper, in a good light.

Generally it is sufficient to make sure that the quantity of lead present is less than 20 parts per million, but if it is necessary to ascertain the exact quantity, comparisons are made with suitable proportions of lead (5, 10, 15, etc., cubic centimeters of the dilute standard lead solution).

*Method II. J. M. Wilkie's Method (J. Soc. Chem. Ind., 1908, 28, 636; Harvey and Wilkie, Chem. and Drug., July 17, 1909).*—7 grm. of the sample are taken for the test, and a separate portion of 2 grm. for the colorimetric comparison, to which the standard lead solution is added. Each is dissolved in about 35 c.c. of hot water, allowed to cool, a few drops of N/10 sodium thiosulphate solution added, and heated to incipient boiling, when the flame is removed. Any ferric iron present is rapidly reduced on cooling. When the solution is water-white, potassium cyanide (1 to 2 c.c. of 10% solution) is added, and then ammonia until the solution just smells of it (excess should be avoided). After diluting in Nessler cylinders, 2 drops of colourless ammonium sulphide solution are added and the colorations compared as described above.

In Germany, the following test, due to W. Klapproth, is used. 20 grm. of the sample are ignited with 0.04 grm. of calcium carbonate in a porcelain crucible. The small residue (which contains all the lead) is dissolved in a few drops of nitric acid, 2 or 3 drops of sulphuric acid added, and the mixture heated to expel the nitric acid. The residue, consisting of lead and calcium sulphates, is dissolved in ammonium acetate solution and the solution filtered from insoluble matter (ferric oxide).

To the clear solution hydrogen sulphide water is added and the resulting brown colouration compared with that of a solution of ammonium acetate in water, containing a known quantity of lead to which hydrogen sulphide water has been added under similar conditions. To make certain that the brown coloration is due to lead and not to copper, some potassium cyanide solution is added, which destroys the brown colour due to copper, but has no effect on that due to lead.

**Other Tests.**—Quantities of 3 grm. of the acid are dissolved in water and submitted to the following tests.

The solution of the pure acid should give no turbidity with barium chloride; nor, after the addition of nitric acid, with silver nitrate. The solution rendered alkaline with ammonia should give no precipitate with ammonium oxalate. The acid should require for titration the calculated quantity of normal alkali, which has been standardised by pure potassium hydrogen tartrate, using phenolphthalein as indicator, under exactly the same conditions of concentration.

**British Pharmacopœia, 1914.**—The requirements of the current Pharmacopœia are as follows for tartaric acid; 1 grm. dissolved in water requires for neutralisation not less than 13.2 c.c. of N/1 solution of sodium hydroxide. It yields no characteristic reaction for copper, iron, or oxalates and not more than the slightest reaction for calcium. *Lead limit* 20 parts per million; *arsenic limit* 1.4 parts per million. 1 grm. dissolved in 50 c.c. of water on addition of 0.5 c.c. of solution of barium chloride does not yield a greater opalescence than 1 c.c. of N/100 solution of sulphuric acid when precipitated under the same conditions (limit of sulphates); ash not more than 0.1%.

**Tartaric acid liquors** are the liquids resulting from the decomposition of calcium tartrate by sulphuric acid. They are of a very complex character, containing: free tartaric acid; foreign organic acids; sulphuric acid, and calcium, potassium, iron and aluminum sulphates; phosphates; and bodies of an indefinite nature. The analytic examination usually includes estimation of the tartaric and free sulphuric acid, with the additional estimation, in some cases, of the total organic acids.

The estimation of the *tartaric acid* is best effected by precipitation as potassium hydrogen tartrate. Potassium acetate is the best reagent for pure liquors, but it is inapplicable in presence of iron or aluminium. Potassium citrate is free from this objection. It is obtained by neutralising citric acid with pure potassium carbonate or hydroxide and is best employed in the following manner:

A quantity of liquor, of 30 to 40 c.c. in volume, as cold as possible, and containing from 2 to 4 grm. of tartaric acid, is treated with a saturated aqueous solution of the citrate, added drop by drop with constant stirring. As soon as the free sulphuric acid is neutralised the precipitate begins to appear in streaks on the sides of the glass.

In presence of much sulphuric acid, a fine precipitate of potassium sulphate will precede the formation of the tartrate, but is readily distinguished therefrom. When the streaks begin to appear, 1 c.c. of citrate solution is added for every grm. of tartaric acid supposed to be present. A great excess should be avoided. Should a gelatinous precipitate be formed, the experiment is repeated with a previous addition of some citric acid. After being stirred continuously for 10 minutes, the precipitate is washed 2 or 3 times with 25 c.c. of a 5% solution of potassium chloride, saturated with potassium hydrogen tartrate. The precipitate is then collected on a small filter and washed with the same solution, until the acidity of the filtrate is only slightly in excess of that of the solution used for washing the precipitate. The filter and precipitate are finally transferred to a beaker, and the amount of tartaric acid present is determined by titration with standard alkali which has been compared with potassium hydrogen tartrate; litmus or phenolphthalein being used as the indicator. The presence of potassium sulphate in the precipitate is of no consequence, as it has no neutralising power.

Sometimes, however, a potassium hydrogen citrate is carried down by the tartrate and obstinately retained. It is best eliminated by dissolving the precipitate in 50 c.c. of hot water, adding 5 grm. of potassium chloride, and cooling the liquid quickly to 15°, stirring continually, and continuing the agitation for 10 minutes. This purified precipitate may be washed with the ordinary washing fluid with great ease, but a correction of 0.5% on the tartaric acid found must be made for unavoidable loss in the process of purification. The filtrate may be tested for citric acid by neutralising it with sodium hydroxide and adding calcium chloride. After prolonged standing in the cold and filtration from a little calcium tartrate, the solution is boiled, when any precipitate will consist of calcium citrate.

Under favourable circumstances, the above method will show from 99 to 100% of the tartaric acid present, but greater differences occur if the proper proportion of citrate is not used. Grosjean concluded that, when an accurate assay of factory tartaric acid liquors is required, a preliminary series of experiments was necessary to ascertain what volume of citrate solution gave a precipitate of maximum acidity. This having been ascertained, a final experiment should be made, using the proper quantity of citrate solution, and washing the precipitate very thoroughly. In presence of much

sulphuric acid, the results have a tendency to be in excess of the truth. From very old bad liquors, potassium alum may be precipitated on adding the citrate solution, owing to the formation of potassium sulphate and the sparing solubility of alum in solutions of that salt. When alum has been precipitated the results will be below the truth, as on washing with the potassium chloride solution a fluid is formed in which potassium hydrogen tartrate is readily soluble. If, on the other hand, an alcoholic washing liquid be substituted, the alum is retained in the precipitate, and increases the final acidity. The difficulty may be avoided by adding phosphoric acid before the citrate solution, but the filtration must be effected immediately after the stirring, or a gelatinous precipitate of aluminum phosphate may be thrown down.

**Racemic acid**, if present, will be estimated as tartaric acid by the above method. *Inactive tartaric acid* is only imperfectly precipitated, owing to the greater solubility of its potassium salt. *Oxalic acid* has been detected in old liquors, but does not interfere with the results.

The estimation of the *free sulphuric acid* in tartaric acid liquors is troublesome, owing to the insolubility of potassium and calcium tartrates in alcohol and the occasional presence of alum. Thus, if mixed solutions of potassium alum and tartaric acid are treated with alcohol, potassium hydrogen tartrate and alum are precipitated, and the liquid contains sulphuric acid, which was not present originally. A similar reaction occurs if calcium sulphate is substituted for the alum. These errors are removed when the quantity of sulphuric acid in the liquor is sufficiently great, and will occur in practice merely in the case of new liquors of bad quality. (For analytical process see p. 711.)

A useful indication of the presence of sulphuric acid in tartaric acid liquors is obtained by treating the liquid with half its volume of a saturated aqueous solution of calcium chloride. A turbidity due to calcium sulphate occurs immediately in a liquor containing sulphuric acid equivalent to 0.8% of brown oil of vitriol, and in 5 minutes when only 0.1% of oil of vitriol is present.

For the estimation of the *total organic acids* in tartaric acid liquors, R. Warington recommends the following method (*J. Chem. Soc.*, 1876, 28, 982): Exactly neutralise a known volume of the liquor with standard alkali hydroxide, evaporate to dryness, and ignite the

residue at a very low temperature till the carbon is nearly consumed. Treat the flask with a known quantity of standard sulphuric acid, heat and decant, and treat the insoluble residue with more standard acid, concentrating, if necessary, to effect solution of the phosphates. Treat the mixed cold concentrated solutions with sufficient potassium sodium tartrate to keep any aluminum in permanent solution, and then titrate the solution with standard alkali and litmus. The amount of standard sulphuric acid neutralised *by the ash* is the exact equivalent of the total organic acid in the liquor taken, and each cubic centimeter of normal acid neutralised represents 0.075 gm. of organic acid, expressed in terms of tartaric acid.

**Lees; Argol; Tartar.**—These are products of the fermentation of grape-juice; they consist largely of potassium hydrogen tartrate and are the materials from which tartaric acid and tartrates are obtained. Their separation is due to the diminished solubility of the tartrates in the alcoholic liquid produced by the fermentation.

Lees is the solid matter collected from the bottom of the vessels in which the grape-juice is fermented.

Its composition is greatly altered by “plastering” the wine. This process consists in adding to the wine an impure calcium sulphate containing some carbonate. “Spanish earth,” a kind of readily decomposed clay, is sometimes employed, although this material is generally free from carbonates. The result is, that in plastered lees the tartrate exists chiefly as the calcium tartrate instead of the acid potassium salt. The total tartaric acid in lees is usually from 24 to 32%. Lees contain from 30 to 40% of indefinite vegetable matter, the remainder being tartrates, sulphates (in plastered lees), ferric oxide, alumina, phosphates and sometimes lumps of plaster.

**Argol, or crude tartar,** is the crystalline crust deposited on the sides of the vessels used for the fermentation. It exhibits some irregularity of composition, the tartaric acid ranging from 40 to 70%, most of it as potassium hydrogen tartrate. Very low argols resemble superior lees, while first-class argols are equal to ordinary refined tartar. The term “argol” is also applied loosely to both tartar and lees. In argol, globules of sulphur are sometimes found; they are due to the sulphur burnt in the casks before introducing the wine.

**Cream of tartar, or refined tartar,** is prepared by boiling crude tartar (argol) with water, filtering, and crystallising the salt from the

clear liquid. The term cream of tartar is derived from the fact that during the evaporation of the liquid the salt collects in white crystalline crusts on the surface of the solution. Cream of tartar consists chiefly of potassium hydrogen tartrate, but contains more or less calcium tartrate, which, though nearly insoluble in pure water, dissolves with moderate facility in a hot solution of potassium hydrogen tartrate. The proportion of calcium tartrate usually present in commercial cream of tartar ranges from 2 to 9%; proportion in excess of 10% may be considered as an adulterant (see a paper by Allen, *Analyst*, 1880, 5, 114). Commercial cream of tartar has been known to be adulterated to a considerable extent, the potassium and calcium sulphates, marble, alum and barium sulphate, starch and calcium phosphate being among the substances used, and potassium hydrogen sulphate has been sold under the name of "tartalie," and employed as a substitute for cream of tartar. It has a higher neutralising power than cream of tartar, and hence is sometimes diluted with potato starch, the mixture being sold under misleading names. Powdered alum has been often sold under the term C. T. S. (cream of tartar substitute).

The total tartaric acid is best estimated by the Goldenberg method.

The *acidity*, on which the percentage of cream of tartar is generally gauged, is ascertained by titrating 5 grm. of the sample with N/1 potassium hydroxide, which has been standardised by titration with 5 grm. of pure recrystallised 100% cream of tartar, dried at 100°, under exactly the same conditions of concentration, using phenolphthalein as indicator. The titration can also be made with 2 grm. of the cream of tartar and N/5 alkali standardised under the same conditions as in the actual test. The observance of exactly similar concentrations for standardisation and the actual test is necessary if exact results are to be obtained, owing to the hydrolysis of the neutral tartrate by water, which causes more alkali to be required (0.1–0.3 c.c.) in dilute solution than in concentrated solution.

To estimate tartaric acid rapidly in cream of tartar, baking powders, etc., F. W. Richardson and J. C. Gregory (*J. Soc. Chem. Ind.*, 1903, 22, 405) and R. O. Brooks (*J. Amer. Chem. Soc.*, 1904, 26, 813) have advocated a polarimetric method.

The requirements as regards *arsenic* and *lead* are the same as for tartaric acid.

**Analysis of Tartar and Argol.**—For the detection of adulterants in cream of tartar, the following tests may be applied.

The sample should be ignited, the residue boiled with water, filtered off, washed, ignited, moistened with ammonium carbonate, gently re-ignited and weighed. The “insoluble ash” thus obtained from genuine cream of tartar consists of the calcium carbonate corresponding to the *calcium tartrate* originally present, and its weight may be calculated to its equivalent of the latter by multiplying it by the factor 1.88. The calcium tartrate thus found should not exceed 10%, or 12% at the outside. Any higher proportion is usually due to adulteration with calcium compounds. Addition of *calcium chloride* is said to have occurred, though improbable, but there are authentic cases of adulteration with *chalk* and *marble*. Allen found 20% of *calcium sulphate* probably added as *plaster of Paris*. In the case of adulterated samples, the proportion of calcium tartrate cannot be deduced with accuracy from the percentage of “insoluble ash.”

The sample is boiled with a moderate excess of pure sodium carbonate and the liquid filtered. A portion of the filtrate is tested for *sulphates* (e. g., calcium sulphate, potassium sulphate and alum) by acidifying slightly with hydrochloric acid and adding barium chloride, and another for *chlorides* by rendering it acid with nitric acid, and adding silver nitrate; traces of sulphates and chlorides may be neglected. The precipitate produced by sodium carbonate should be rinsed off the filter and treated with dilute hydrochloric acid. Any insoluble residue may consist of *sand* or *barium sulphate*. Both the chemical and microscopical characters may be employed to distinguish these, and to determine whether the latter adulterant is crystalline or amorphous.

The presence of *alum* is indicated by the detection of a notable quantity of sulphates, and the presence of aluminum oxide in the insoluble ash. Aluminum hydroxide cannot be precipitated by adding ammonium hydroxide to the original solution of the substance, owing to the presence of tartrate; but it may be detected by neutralising the hot solution of the sample with sodium hydroxide, and boiling the liquid with a little acetic acid and excess of sodium phosphate. Any aluminum present will be thrown down as phosphate, tartrates having scarcely any solvent action on the precipitate at the temperature of ebullition, and in presence of excess of phosphoric acid.

Alum may be dissolved out of cream of tartar by treating the finely powdered sample with a cold, saturated, aqueous solution of potassium hydrogen tartrate, containing 5% of potassium chloride.

**Starch** is easily detected by microscopic examination and the iodine test. For estimation see under "Starch."

**Calcium phosphates** are detected and estimated by treating 0.5 gm. with excess of moderately strong nitric acid, and precipitating with ammonium molybdate in the usual way.

**Analysis of Crude Tartars.**—The examination may be made either to estimate the potassium hydrogen tartrate present or the total tartaric acid that will be yielded by the sample.

**Estimation of Potassium Hydrogen Tartrate.**—Oulman's method (Lunge, *Chem. Techn. Unters. Meth.*, Vol. 3):

3.76 gm. of the finely powdered sample are put into a 1000 c.c. flask with 750 c.c. of water, boiled for, at most, 5 minutes, made up to the mark, cooled, again made up to the mark, mixed and 500 c.c. of filtrate collected through a dry filter. This filtrate is evaporated to dryness in a porcelain basin on the water-bath. While the dry mass is still warm, it is moistened with 5 c.c. of water, cooled and 100 c.c. of alcohol added, the mixture thoroughly stirred and allowed to stand for 30 minutes. The alcohol is then decanted through a dry filter, and the last portion drawn through with the pump. Any acid potassium tartrate on the filter is washed back into the evaporating basin with boiling water, the solution diluted with water to make 100 c.c. and titrated with N/5 alkali. 0.2 c.c. should be added to the titration figure for correction.

**Total Tartaric Acid.**—The following process for analysis of tartar is designated "Goldenberg 1907" (*Zeitsch. anal. Chem.*, 1908, **47**, 57), and was approved at the 7th *International Congress of Applied Chemistry*, London, 1909. It is now in general use.

A weighed amount of the sample (6 gm. if the tartaric acid yield is likely to be above 45%; 12 gm. if below that amount) is treated with 18 c.c. of hydrochloric acid (sp. gr. 1.1) for 10 minutes. The whole mass is then rinsed into a 200 c.c. measuring flask, made up to the mark with distilled water, shaken well and filtered through a dry filter into a dry flask. 10 c.c. of potassium carbonate solution (66 gm. of absolute carbonate in 100 c.c.) are placed in a 300 c.c. beaker and 100 c.c. of the filtered liquid added. The capacity of the pipette by which this volume is measured must correspond exactly

with that of the flask. The mixture is brought to boiling and kept at that point for 20 minutes, until the calcium carbonate has separated in crystalline form. The liquid and precipitate are washed into a 200 c.c. flask, cooled, made up to the mark, shaken well and filtered through a dry filter. A volume of 100 c.c. of the filtrate is placed in a porcelain basin or Jena beaker and evaporated on the hot plate to 15 c.c. and, while the liquid is hot, 3.5 c.c. of glacial acetic acid are added gradually and with constant stirring, which is continued for 5 minutes after all the acid has been added. After 10 minutes' standing, 100 c.c. of alcohol (95%) are added, and the liquid stirred for another 5 minutes, and after standing for another 10 minutes the liquid is filtered by the aid of a pump and the precipitate washed with alcohol until the washings are no longer acid (see below). The filter and precipitate are transferred by the aid of 200 c.c. of hot water into a porcelain basin, the liquid brought to boiling and titrated with N/5 alkali and neutral litmus paper. The alkali must be standardised with the same paper, using pure potassium hydrogen tartrate. As the volume of undissolved matter is disregarded in making up the dilutions, an allowance must be made. It is agreed that for samples yielding less than 45% of acid, 0.8 should be deducted; for samples yielding from 45% to 60%, 0.3 should be deducted; for those yielding 60% to 70%, 0.2 should be deducted; for yields over 70% no deduction is made.

To control the washings it is advised that 30 c.c. of the alcohol that is to be used should be titrated with standard alkali, using phenolphthalein, and that the washing should be continued until 30 c.c. of the filtrate require the same amount of standard alkali (with phenolphthalein) to give the colour that was produced in the test of the original 30 c.c.

Porcelain dishes marked with a ring at the volume of 15 c.c. can be obtained.

*Warington's Method for Wine Leecs.*—Place 8 grm. of the sample in a beaker, moisten with water and heat on water-bath about 5 minutes. Add 2 grm. of potassium oxalate and heat the mixture 15 minutes on water-bath. While hot *almost* exactly neutralise with potassium hydroxide solution (3.5% solution), taking care not to neutralise completely, and avoiding an excess of alkali. The quantity of alkali used is about 0.5% short of that required for complete neutralisation, as ascertained by a separate experiment

(see below). After neutralisation in this way, heat on water-bath about 30 minutes and filter, preferably on filter pump, using porcelain plate 2.5 cm. diameter. (For difficulties experienced with slow filtering material see Grosjean, *Trans.*, 1879.) Wash with 10 lots of water, using 3 c.c. at a time. This should be sufficient, and the filtrate should have a volume of about 50 c.c. *Make to this volume* either by addition of water or by evaporation. Add 5 gram. potassium chloride and 2.5 gram. citric acid, stir well *continuously* during 10 minutes and allow the beaker to stand. Filter off the potassium hydrogen tartrate with the aid of a pump, wash with a 10% solution of potassium chloride saturated with potassium hydrogen tartrate, of which the acidity has been ascertained by N/10 alkali. When the acidity of the washings is the same as that of the washing solution, dissolve the precipitate in hot water and titrate with N/10 potassium hydroxide.

*For Tartars.*—Use 3 gram. and proceed as above.

*Preliminary Determination of Acidity.*—Extract exactly 3 gram. of the sample by boiling with water, decanting, again boiling and again decanting. The residue is transferred to filter paper and thoroughly washed until the washings are no longer acid, titrated with N/10 alkali or with 3.5% potassium hydroxide solution, neutral litmus paper being used.

The following special precautions applicable to the above processes are taken from Rasch's book (*Die Fabrikation der Weinsäure*):

The sample must be ground very fine. The alcohol and water must be neutral to the indicators employed. The potassium carbonate should be pure, especially free from iron and aluminum. The procedures must be at ordinary temperature unless otherwise directed. The evaporation of the solution containing potassium carbonate must not be carried too far and the treatment with acetic acid must be while the liquid is hot. These conditions are necessary to secure crystalline precipitates. The acetic acid strength must not be below 98%.

The washing with alcohol must be carefully carried out. It is best to stir the precipitate with the stream from the jet of the wash bottle, and then wash the funnel margin above the filter. Usually it will be sufficient to fill the filter in this manner three-fourths full five successive times.

The standard potassium hydroxide must be free from carbonate and be accurately titrated with pure potassium hydrogen tartrate, using exactly the same kind of litmus paper that is used in the analysis.

**Analysis of Tartaric Acid Raw Material.**—Although the analysis of raw material used in the manufacture of tartaric acid and its salts (cream of tartar, Rochelle salt, tartar emetic) is now generally made by the "Goldenberg 1907 Method" (page 724), the so-called "London Method" has been adopted for purposes of sale of tartars (argols, vinaccia tartar) and tartrate of lime in the London market; lees are still purchased in the London market on analyses made by the Goldenberg method.

**London Method for Argols and Similar Tartars (Alambic Tartars).**—1.5 grm. of the finely divided sample are washed into a 350 c.c. beaker (tall form) with 10 c.c. of a solution of potassium carbonate (40 grm.  $K_2CO_3$  in 100 c.c. of water); about 2 grm. of purified animal charcoal and water to make about 25 c.c. in all are added. The mixture is boiled gently for 20 minutes. The solution is then filtered by means of a vacuum apparatus, into a 250 c.c. beaker (tall form), the residue being washed with hot water until a total volume of 150 c.c. is obtained. 5 c.c. of hydrochloric acid (sp. gr. 1.1) are added and the solution concentrated to about 15 c.c. on a hot plate, avoiding actual boiling. 4 c.c. of glacial acetic acid are then added and the mixture well stirred for 5 minutes. When the mixture is cold 100 c.c. of 92 to 95% alcohol are added and the mixture is again stirred for 5 minutes. After the precipitated potassium hydrogen tartrate has stood for  $\frac{1}{2}$  hour, it is filtered off on a filter-pump plate and washed with alcohol, exactly as in the Goldenberg method, until the washings are free from acidity (see page 724). The precipitate is dissolved in 200 c.c. of hot water and the solution is titrated, whilst boiling, with N/5 sodium hydroxide.

Phenolphthalein is used as indicator, and as the solutions are frequently highly coloured, it is used as an outside indicator on a clean white glazed tile. The sodium hydroxide used should be standardised with pure dry recrystallised potassium hydrogen tartrate under similar conditions.

For tartrate of lime the same method is employed, but the animal charcoal can be omitted.

### London Method for Vinaccia and High-grade Tartars.

1. **Tartaric Acid Present as Bitartrate.**—5 grm. of the sample are washed into a 400 c.c. beaker with 250 c.c. of hot water and 15 c.c. of N/1 sodium hydroxide. After boiling for 10 minutes, the titration is completed with N/1 sodium hydroxide using phenolphthalein as indicator.

2. **Tartaric Acid Present as "Tartrate of Lime" (Calcium Tartrate).** 2 grm. of the sample are ignited in a covered platinum dish or crucible at a low temperature. When charred, the residue is washed into a beaker with 5 c.c. of hydrogen peroxide (10 volume), 30 c.c. of normal hydrochloric acid are added and the solution well boiled to destroy the excess of peroxide. Phenolphthalein is then added and the solution titrated back with N/1 sodium hydroxide. In this way the number of cubic centimeters of N/1 HCl required to neutralise the ash of 1 grm. is obtained. From this is deducted the number of cubic centimeters of N/1 sodium hydroxide required to neutralise the bitartrate present in 1 grm., as found by method 1, and the percentage of tartaric acid in the form of calcium tartrate is calculated from the difference (each cubic centimeter = 0.075 grm. of tartaric acid in the form of calcium tartrate).

**Other Methods of Estimating Tartaric Acid.**—It is generally recognised that the Goldenberg method, using litmus as indicator, gives slightly low results for tartaric acid when, as is usual in the case of raw material such as lees and crude tartars, phosphates of aluminium and iron are present or certain other substances such as malic acid or gummy impurities accompany the tartaric acid.<sup>1</sup> As, however, the presence of such impurities in tartaric acid raw material causes considerable loss of the acid during its manufacture, the Goldenberg method represents, in the case of the lower grade material, probably the fairest means of arriving at the value of such consignments. It must be remembered, however, that the Goldenberg process is a conventional one, and may, when absolute results are required, give rise, in presence of the impurities enumerated above, to some considerable degree of error, ranging from 0.5 to 5%

<sup>1</sup> Compare Heczko, *Zeit. anal. Chem.*, 1911, 50, 12 and 73. The objections which have been raised to the Goldenberg method since its adoption are based on facts which were well realised by the manufacturers and committees responsible for its acceptance as a practical test of the value of raw material on the grounds given above. The proposal by Ordonneau (*Bull. Soc. Chim.*, 1909 [iv] 7, 1034), that phenolphthalein should be used as indicator, would lead to higher prices being paid for low-grade material, the tartaric acid of which cannot be completely extracted owing to the presence of alumina, phosphoric acid, etc.

of the tartaric acid present, according to the proportion and nature of the impurities present.

Various other processes of estimating tartaric acid have therefore been suggested, none of which has yet been adopted for commercial purposes. A brief description of some of the most promising of these methods is here appended, as in certain special cases such methods might find a useful application.

**Chapman and Whitteridge's Method** (*Analyst*, 1907, 32, 163).—In this method, the tartaric acid is precipitated from its solution as bismuth tartrate, the latter being practically insoluble in dilute acetic acid; the barium tartrate is titrated, subsequently, in sulphuric acid solution, with potassium permanganate. The process is carried out as follows: A known weight of the substance to be analysed, containing about 0.1 grm. of tartaric acid, is dissolved in 40 c.c. of water; the solution is neutralised with either sodium hydroxide or acetic acid, as the case may require, and is then heated nearly to boiling. 15 c.c. of bismuth reagent (prepared by dissolving 30 grm. of crystallised bismuth nitrate in 20 c.c. of glacial acetic acid, diluting the solution to a volume of 300 c.c. with water, and filtering, if necessary) are added, the mixture is stirred vigorously for a few minutes, and the precipitate is collected on a filter and washed well with boiling water. The precipitate is then dissolved off the paper with about 20 c.c. of hot 10% (by volume) sulphuric acid, a further 30 c.c. of the same acid being used to wash the paper. A 1% potassium permanganate solution is then run into the hot acid filtrate until a slight excess, not exceeding 0.5 c.c., is present, and this is titrated back with oxalic acid solution containing 19.9 grm. of the pure crystallised acid per litre. As the result of many determinations, it has been found that 0.1 grm. of tartaric acid corresponds with 14 c.c. of the permanganate solution. For complete precipitation, it is well to add 1.5 times the quantity of bismuth reagent theoretically necessary. The method gives accurate results in the case of Rochelle salt, crude tartars, Seidlitz powders, and baking powders. Alum and succinic acid, if present, have no influence on the accuracy of the results, but the method cannot be applied to the determination of tartaric acid in the presence of citric, oxalic, and malic acids.

**Beys' Method** (*Bull. Soc. Chim.*, 1910, 7, 697).—Between 1 and 2 grm. of the material to be analysed is heated for half an hour on

the water-bath with an equal weight of sulphuric acid diluted with 20 times its volume of water. When the liquid is cool, 5 times its volume of alcohol and 10 times its volume of ether are added, and the liquid filtered. The residue is washed with a mixture of 1 part of alcohol with 2 parts of ether. Some phenolphthalein is added to the filtrate, which is neutralised with potassium hydroxide dissolved in 90% alcohol. The amount of the latter is noted, and 5 to 10 c.c. more are added. The whole is heated till the ether begins to boil off, when it is allowed to cool. The clear liquid is poured off and excess of glacial acetic acid added. The crystals left behind are heated with 12 to 15 c.c. of water and 5 c.c. of glacial acetic acid are added, causing the precipitation of the greater portion of the potassium hydrogen tartrate. 30 c.c. of 96% alcohol are added, which is about the quantity required to make the alcoholic strength of the mixture 65%. After shaking for a few minutes and standing for an hour the precipitate is filtered off, washed first with 15 c.c. of 65% alcohol, then with 96% alcohol. The other precipitate produced by the addition of acetic acid (see above) is washed with 96% alcohol. Both precipitates are dissolved in the same quantity of boiling water. The liquid is then titrated as in the Goldenberg method, with the use of phenolphthalein as indicator.

**Kling's Method** (*Compt. rend.*, 1910, 150, 616).—The errors which arise in the usual method of estimating tartaric acid by precipitation of its acid potassium salt are said to be avoided by precipitating the acid as calcium racemate,  $\text{Ca}(\text{C}_4\text{H}_4\text{O}_6)_2 \cdot 8\text{H}_2\text{O}$ , and titrating a solution of this with potassium permanganate. The details of the method are as follows: 25 c.c. of a solution of *d*-tartaric acid (containing 3 to 4 grm. per litre) are added to 100 c.c. of water, 25 c.c. of a solution of *l*-Seignette salt (16 grm. per litre: free from *d*-salt) and 20 c.c. of a solution of pure calcium acetate (30 grm. per litre). The precipitate is collected, washed and dissolved in 20 c.c. of hydrochloric acid (40 grm. per litre). The solution is diluted to 150 c.c. and added to 40 c.c. of a solution of sodium acetate (10%) and of calcium acetate (1%) and boiled. After cooling, the racemate is collected, washed with water, redissolved in 10% boiling sulphuric acid and titrated at the b. p. with a solution of permanganate (containing about 16 grm. per litre) of which the titre has been determined by means of pure bitartrate. The method gives good results and can be used for the estimation of tartaric acid in wines.

In a later paper Kling and Florentin (*Eighth Int. Congress App. Chem.*, 1912, Sect. I, 237) have modified this process so as to determine with a considerable degree of accuracy tartaric acid contained in metallic salts, tartars, and lees.

The solutions required are: (a) a solution of di-ammonium citrate, containing 50 grm. of the salt per litre; (b) a solution containing 20 grm. of pure ammonium *l*-tartrate free from the *d*-compound (see below, page 710), and 5 or 6 c.c. of formaldehyde (as preservative) per litre; (c) a solution prepared by dissolving 16 grm. of chemically pure calcium carbonate in 120 c.c. of glacial acetic acid and diluting to 1 litre; (d) dilute hydrochloric acid containing 40 grm. of the 22° Bé. acid per litre; (e) a solution prepared by dissolving 5 grm. of calcium carbonate in 20 grm. of acetic acid, adding 100 grm. of sodium acetate and diluting to 1 litre; (f) a solution of potassium permanganate containing 16 grm. per litre. The permanganate is standardised with a solution of tartaric acid of known titre, determined with N/10 potassium hydroxide. In the estimation of tartaric acid, in the presence of interfering metals or not, the solution is diluted to a volume of 150 c.c., 10 to 15 c.c. of solution *a* are added, and then successively 25 c.c. of solution *b* and 20 c.c. of solution *c*. The mixture is agitated and allowed to stand for several hours (12 hours if appreciable quantities of interfering metals are present). After being filtered off and washed with cold water, the precipitated racemate is dissolved in 20 c.c. of the dilute hydrochloric acid (solution *d*), the solution is diluted to 150 c.c., 40 to 50 c.c. of solution *e* are added, the mixture is heated to about 80° C. and then allowed to cool for several hours. The resulting precipitate is filtered off, washed, redissolved in hot, dilute sulphuric acid (10% by volume) and titrated at the b. p. with the permanganate solution.

This process was also applied by Kling and Gelin (*Eighth Int. Congress App. Chem.*, 1912, Sect. I, i, 251) to solutions prepared by heating alcoholic solutions of tartaric acid in sealed tubes and containing known amounts of tartaric acid, the latter being present in the free state and in the form of acid and neutral ethyl tartrates. The amount of tartaric acid corresponding to the total acidity being found by titration with N/10 potassium hydroxide, the free acid present was estimated by the racemate method in a second portion of the liquid. A third portion was hydrolysed with alkali in very dilute solution, the racemate process being then applied to

estimate the total (free and combined) acid in the liquid. The results thus obtained were concordant and corresponded with the amounts of tartaric acid known to be present. The process is stated to be applicable to wines and other fermented liquors.

According to Kling and Florentin (*Ann. Falsif.*, 1912, 5, 518), the racemate method for the estimation of tartaric acid was found to yield accurate results even in the case of materials containing considerable quantities of iron and aluminium oxides. The results obtained are, in certain cases, somewhat higher than those found by the usual methods, this being due to the fact that the racemate-citrate method gives the whole of the tartaric acid present.

Kling and Gobert (*Bull. Assoc. Chim. Sucr.*, 1911, 28, 760) have applied the racemate method described above to substances which are strongly acid but contain tartaric acid in only small amount, such as vinegar, cider and perry. Fully satisfactory results were obtained, whereas those found by the methods generally used were frequently much too low. It is emphasised that in using the racemate method it is very necessary to use pure *l*-tartrate and in purifying the commercial product from the *d*-compound the following procedure has been found convenient: 20 grm. of ammonium *l*-bitartrate are dissolved in 900 c.c. of water; 20 c.c. of this are diluted to 200 c.c. with water, and to this liquid 10 c.c. of the solution of calcium acetate (*supra*) added. After half an hour, the precipitate is filtered off, washed, dried and calcined, and from the weight of residue the amount of calcium acetate which should be added to the 880 c.c. to separate all the *d*-tartaric acid is calculated. This addition being made, the liquid is allowed to stand for 12 hours, filtered and diluted to a litre, a little formaldehyde being added as preservative. This constitutes the solution *b* (*supra*).

**Estimation of Tartaric Acid in the Presence of Malic and Succinic Acids (Wines and Fruit Juices).**—The simple evaporation method proposed originally by Pasteur, and modified by Reboul, was found by Mestrezat (*Ann. Chim., Anal.*, 1908, 13, 433) to give accurate results in the estimation of tartaric acid in wines, and is considered to be a better method than the official (French) process. The author prefers to work as follows: 50 c.c. of the wine are evaporated to the consistency of a syrup; when cold the residue should be semi-fluid, but, if the evaporation has been carried too far, a few drops of water may be added. The basin containing the residue is then

placed aside for at least 4 days; after this time, the mass is taken up with a small quantity of 40% alcohol saturated with potassium hydrogen tartrate, and the solution is carefully decanted from the crystals. The latter are washed with the same solution, by decantation and then titrated. Results are given showing that concordant analyses may be made by the method and that the presence of malic, succinic and acetic acids is without influence on the determinations.

Gowing-Scopes (*Analyst*, 1908, **33**, 315) has investigated the method proposed by J. von Ferentzy (*Chem.-Zeit.*, 1907, **31**, 1118), which is based on the insolubility of basic magnesium tartrate in 50% alcohol and finds that tartaric acid may be accurately estimated in fruit juices by this method, and also in wines, if the tannic acid be first removed. More accurate results are obtained if the basic magnesium tartrate is titrated with permanganate than when it is ignited as recommended by Ferentzy. The following are the details of the process: A solution of the substance under examination, containing not less than 0.05 grm., and not more than 0.10 grm., of tartaric acid, is taken for the estimation; if the bulk of the solution be large, or if alcohol be present, the solution is evaporated to about one-half of the original volume. To the cooled solution is then added an equal volume of absolute alcohol and the precipitate which forms in the case of fruit juices and wines is filtered off and washed with 50% alcohol (by volume). 10 c.c. of ammonia and 10 c.c. of absolute alcohol are added to the filtrate, any precipitate which forms being again removed by filtration and washed with 50% alcohol. To the filtrate thus obtained are added 10 c.c. of "magnesia mixture" and 10 c.c. of absolute alcohol, the mixture being thoroughly stirred meanwhile. After standing overnight, the solution is filtered through double filter papers, the precipitate is washed with 50% alcohol and is then dissolved off the filter with boiling water, about 400 c.c. being used. The solution is evaporated to a volume of about 200 c.c., or until all the alcohol has been removed, then cooled and diluted to a volume of about 400 c.c., after the addition of 10 c.c. of concentrated sulphuric acid. The solution is next heated to a temperature of 90° C., and potassium permanganate solution is run in a little at a time, until 2 or 3 drops have been added in excess; this excess is then titrated back with oxalic acid solution. The potassium permanganate solution should contain 6.9745 grm. of the

salt per litre; 1 c.c. corresponds with 0.0050 grm. of tartaric acid. The oxalic solution should be of equivalent strength.

The following method of estimating malic and tartaric acids in the same solution has been proposed by Dunbar (*U. S. Dept. Agric., Bureau of Chem., Circular No. 105, Oct. 4, 1912*): Solutions of both malic and tartaric acids when treated with uranyl acetate show an increased rotation, which, within certain limits, is proportional to the concentration (see *J. Soc. Chem., Ind.*, 1911, 1177, 1407, compare page 98) both acids reduce definite quantities of potassium permanganate in alkaline solution and form oxalic acid quantitatively. Hence it is possible to calculate the amounts of malic and tartaric acids in a solution, knowing the total change in the rotation on treatment with uranyl acetate, and also either the amount of potassium permanganate reduced or the amount of oxalic acid formed. The details of the method are as follows: 85 c.c. of the solution are rendered slightly alkaline with sodium hydroxide, and 5 grm. of sodium acetate are dissolved in the mixture, which is then made acid with citric acid and diluted to 100 c.c. About 30 c.c. of this solution are shaken for 3 hours with from 2 to 3 grm. of uranyl acetate; if all the latter dissolves more must be added. After filtration, the solution is polarised in a 200 mm. tube, and the reading in degrees Ventzke observed calculated to the basis of the original solution. If optically active substances other than malic and tartaric acids are present, 50 c.c. of the original solution must be treated with an excess of dry powdered lead acetate, filtered, the excess of lead removed from the filtrate by means of anhydrous sodium sulphate, and the solution polarised. The difference between the two readings will give the rotation due to the uranium complexes of the two acids. The quantity of permanganate reduced by the mixed acids is determined by rendering a portion of the solution alkaline with sodium hydroxide, adding an excess of about 3 grm. of the latter and heating the mixture with 50 c.c. of a 1.5% permanganate solution on the water-bath for 1 hour. The mixture is then acidified with sulphuric acid and the excess of permanganate titrated with oxalic acid solution. In the presence of other substances which reduce permanganate but do not form oxalic acid, the amount of the latter may be determined in the usual way by titration with permanganate; 1 c.c. of 1.5% potassium permanganate solution is equivalent to 0.0299 grm. of crystallised oxalic acid. Let  $x$  be the grams of malic acid, and  $y$  the grams

of tartaric acid, per 100 c.c. of solution,  $a$  the polarisation in degrees Ventzke in a 200 mm. tube after treatment with uranyl acetate, and  $b$  the grams of potassium permanganate required to oxidise 100 c.c. of the solution, then  $x = -0.0185a + 0.1720b$ , and  $y = 0.0248a + 0.2436b$ . If  $c$  equals grams of oxalic acid yielded by 100 c.c. of the solution then  $x = -0.020a + 0.0233c$ , and  $y = 0.23a + 0.331c$ . The data taken into account in these equations are: 1 grm. of malic acid in 100 c.c. will show a rotation of  $-27.77^\circ$  V. after treatment with uranyl acetate, and 1 grm. of tartaric acid per 100 c.c. a rotation of  $19.61^\circ$  V.; 1 grm. of malic acid reduces 2.8297 grm.  $\text{KMnO}_4$  and 1 grm. of tartaric acid reduces 2.1062 grm. In the case of fruit juices and solutions containing sugars, 50 c.c. of the solution are mixed with about 150 c.c. of 95% alcohol and the mixed acids are precipitated with lead acetate; the precipitate is collected on a filter, washed with 80% alcohol until free from sugars, then decomposed with dilute sulphuric acid, the lead sulphate is removed by filtration and the alcohol by evaporation and the solution of acids so obtained treated as above described.

Another method (P. Dutoit and M. Duboux, *Bull. Soc. Chim.*, 1913, [IV], 13, 832) for the separate estimation of tartaric, malic and succinic acids when occurring together depends on the fact that, under certain defined conditions (faint acidity and relatively low concentration of alcohol), all three acids are precipitated by lanthanum nitrate, whereas other conditions, also closely defined, determine the precipitation of tartaric acid alone, or of tartaric and malic acids.

*Calcium Tartrate Analysis.*—The following modification of the "Goldenberg 1907" method (see page 724) was adopted for calcium tartrate at the Seventh International Congress of Applied Chemistry (London, 1909).<sup>1</sup>

6 grm. are always to be taken and the potassium carbonate solution is added to the 100 c.c. of the hydrochloric acid solution, drop by drop by means of a pipette, at such rate as to require in all about 5 minutes. The mixture is boiled for 20 minutes longer as directed above. The modified procedure is to avoid the occlusion of calcium tartrate in the calcium carbonate.

For the estimation of calcium carbonate in calcium tartrate, the carbon dioxide that the sample will yield must be weighed directly.

<sup>1</sup> Special communication from Mr. W. A. Davis.

*Commercial Cream of Tartar.*—Allen suggested (*J. Soc. Chem. Ind.*, 1896, 15, 681) the following methods:

1. Dissolve 1.1881 grm. of the sample, free from moisture, in hot water and titrate with N/10 alkali, phenolphthalein being used as an indicator. In the absence of acid potassium sulphate and tartaric acid, each cubic centimeter of alkali represents 1% of acid potassium tartrate.

2. Ignite 1.881 grm. for 10 minutes, boil with water, filter and wash the residue.

(a) Titrate the filtrate with N/10 hydrochloric acid and methyl-orange. With pure tartar, the quantity of acid used will equal that consumed in the previous titration with alkali. Each cubic centimeter of the deficiency of acid is equivalent to 0.36% of calcium sulphate, or 0.72% of potassium hydrogen sulphate. Any excess of acid added points to the presence of potassium tartrate, each cubic centimeter representing 0.6% thereof. If the titrated liquid be treated with barium chloride, the barium sulphate will be a measure of the calcium sulphate or potassium sulphate present.

(b) The carbonaceous residue is ignited, dissolved in 20 c.c. of N/10 acid, filtered from any insoluble residue, and the filtrate titrated with N/10 alkali. Each cubic centimeter corresponds to 0.50% of calcium tartrate or 0.36% of calcium sulphate (anhydrous).

The following processes, described by Rasch, are included in Lunge's *Chemische-Technische Unters. Methoden*, being for analysis required in the routine of tartar works. The potassium carbonate solution required contains 5 grm. of the pure salt in 100 c.c. of solution. Phenolphthalein is used as indicator and N/10 potassium hydroxide for titration.

*Tartaric Liquors.*—10 c.c. are boiled with 40 c.c. of the potassium carbonate solution for a short time, made up to 200 c.c., filtered through a dry filter, 10 c.c. of the filtrate mixed with 3 c.c. of glacial acetic acid and 100 c.c. of alcohol and the precipitate titrated. The number of cubic centimeters used multiplied by 30 will give grams of tartaric acid yield per 1000 c.c. of liquor.

*Old Mother-liquors.*—10 c.c. of this are mixed with 60 c.c. of potassium carbonate solution, boiled, cooled, made up to 200 c.c. filtered through a dry filter, 20 c.c. of the filtrate mixed with 5 c.c. of glacial acetic acid and 100 c.c. of alcohol and the precipitate titrated.

The cubic centimeters used multiplied by 15 will give grams of tartaric acid per 1000 c.c. of liquor.

*Residuums.*—300 grm. are treated in a porcelain basin with 25 c.c. of hydrochloric acid (sp. gr. 1.1) and 500 c.c. of water, the mixture being heated to boiling with constant stirring. A portion of the liquid is filtered, 50 c.c. of the filtrate mixed with 5 c.c. of glacial acetic acid and 130 c.c. of alcohol. The precipitate is titrated. Each 5 c.c. required will be approximately equal to 0.1% of tartaric acid in the material.

*Mother-liquor from Calcium Tartrate Precipitates.*—200 c.c. are evaporated to 50 c.c., boiled for a few minutes with 10 c.c. of the potassium carbonate solution, made up to 100., c.c. filtered through a dry filter, 60 c.c. of the filtrate mixed in a measuring flask with 10 c.c. of hydrochloric acid (sp. gr. 1.1) and alcohol added to make a volume of 180 c.c. The mixture is shaken, filtered promptly through a dry filter, and the following are added in succession to 150 c.c. of the filtrate. 10 c.c. potassium carbonate solution, 5 c.c. glacial acetic acid, and 100 c.c. of alcohol. The mixture is well shaken and allowed to stand for 24 hours.

The precipitate is titrated. Each 10 c.c. used will be equivalent to 1.5 grm. tartaric acid in 1000 c.c. of the liquor.

*Free Sulphuric Acid in Liquors.*—20 c.c. of the liquor are made up to 200 c.c. with alcohol, allowed to stand overnight, filtered through a dry filter, 100 c.c. of the filtrate cleared of alcohol and precipitated with barium chloride as usual.

*Detection of Lead.*—The following process is from a description furnished by W. A. Davis.

10 grm. of tartaric acid are dissolved in about 20 c.c. of distilled water, the solution filtered if necessary and placed in a tall, narrow cylinder of colourless glass marked at 100 c.c. A solution of hydrogen sulphide (made by passing the gas through water for at least 2 hours before using) is added in amount sufficient to make 100 c.c. and after 10 minutes the colour of the solution is noted. If no colour is produced lead is absent, or at least below 0.0005%. This is the case with the best product.

A slight bluish turbidity represents about 0.00075%.

A decided blue-yellow or grey represents 0.001%.

A brown tint may be due to either iron or lead, but the latter is usually distinguished by the blacker tint seen when the liquid is held against a white background.

The detection of lead is materially influenced by the presence of iron. Freshly precipitated ferric hydroxide will, by adsorption, carry down lead hydroxide. The procedure under such conditions has been investigated by J. M. Wilkie (*J. Soc. Chem. Ind.*, 1909, **28**, 637) who finds that the usual method of preventing precipitation of iron by adding potassium cyanide succeeds only when the iron is in the ferrous state. As a result of many experiments, Wilkie gives a special process. He uses sodium sulphide as the final precipitate, but W. A. Davis prefers freshly-made colourless ammonium sulphide, prepared by diluting 2 c.c. of 0.880 ammonia to 10 c.c. and saturating with well-washed hydrogen sulphide. Standard lead solutions may be conveniently made from a strong solution of pure lead (5 grm. in nitric acid, evaporated to remove all but a small amount of the acid and made up to 1000 c.c.). This strong solution keeps fairly well. For use portions of it are diluted 100 times and applied in the usual manner for colour comparisons.

10 grm. of cream of tartar are heated with about 50 c.c. of water, and ammonia added until all the potassium hydrogen tartrate is dissolved. If the solution is coloured it must be treated with purified animal charcoal and filtered. The liquid is diluted to 100 c.c., as above noted, and 3 drops of ammonium sulphide added. If the reagent is yellow, allowance must be made for this tint.

If no coloration is produced by the reagent, lead is below 0.0005%.

A slight brownish-yellow shows about 0.00075%.

A clear brown tint, about straw coloured, shows about 0.001%.

Copper is shown by the blue tint imparted to the ammonia solution before the sulphide is added; iron shows a dark green precipitate or a green tint. These tints mask the lead reaction and in such cases a few drops of potassium cyanide solution must be added to the alkaline solution before filtering, and the above procedure followed.

*For the separation* of ordinary tartaric acid, mesotartaric acid, and the racemic association of two active forms, Hollemann (*Rec. Trav. Chim.*, 1898, **17**, 66) devised the following method: The aqueous solution of the free acid is evaporated in the water-bath until crystallisation begins, and the liquid is allowed to stand in a cool place for 24 hours. Racemic acid separates and the crystals may be carefully drained, dried, and weighed. The mother liquor is diluted to 20 c.c.; one-half of this exactly neutralised by potassium hydroxide, the other

half added, and the mixture allowed to stand overnight. Potassium hydrogen tartrate separates quantitatively, and can be collected, dried, and weighed. The filtrate is treated with ammonia, then slightly acidified with acetic acid, boiled, and calcium chloride solution added. The calcium mesotartrate is thrown down.

*Fermentation Test for Lees.*—Rasch states (Lunge, *Chem. Tech. Unters. Meth.*, Vol. 3) that it is sometimes advisable to ascertain if lees contains bacteria likely to cause fermentation, and recommends the following test: 40 grm. of the sample are stirred with some water in a 400 c.c. beaker, 50 c.c. of 10% calcium chloride solution added, the solution neutralised accurately with milk of lime, the beaker filled with water, and the mixture kept at 35° for 24 hours. Good, well-dried lees, will not show appreciable fermentation.

**Tartrates.**—Tartaric acid contains 4 atoms of replaceable hydrogen but only 2 of these are in the true acid-forming position, hence the acid is dibasic and with members of the potassium group forms two series of salts, tartrates and hydrogen tartrates, the latter being often erroneously called "bitartrates." Few of the salts are soluble in water, and all are insoluble in alcohol. The salts of the members of the potassium group unite readily with those of some of the other groups to form double tartrates which are not decomposed on adding strong hydroxides. In this way, the addition of sodium potassium tartrate to copper sulphate solution will prevent entirely the precipitation of copper hydroxide on adding sodium hydroxide. This mixture is known as Fehling's solution. The analysis of these double tartrates is described on page 741.

**Potassium Tartrates.**—The most important of these salts is the potassium hydrogen tartrate. This is the principal constituent of tartar, argol, and wine-lees, and is of importance in the pure state as a source of tartaric acid and as a form for the estimation of that substance.

Pure potassium hydrogen tartrate may be conveniently prepared by dividing a solution of tartaric acid into 2 equal parts, neutralising one portion with potassium carbonate, and adding the other. The product may be purified by recrystallisation from hot water.

It forms colourless crystals, is soluble in 240 parts of water at 10°, 180 at 20°, and in about 15 parts of boiling water. In alcohol it is much less soluble. It requires (at 15°) 400 parts of a liquid containing 10.5% of alcohol, and for 50% alcohol about 2000 parts for

solution. In still stronger alcohol it is practically insoluble. The presence of glucose does not affect its solubility in water or weak alcohol; but some salts and acids have great influence. This is shown by the following table by Warington, in which the effect of water containing equivalent quantities of acids is given. For comparison with them, experiments were also made with solutions containing equivalent amounts of acetic and citric acids neutralised by potassium hydroxide. All the experiments were made at 14°:

Solvent	Grm. of acid or salt in 100 c.c. of solvent	Grm. of tartrate dissolved by 100 c.c. of solvent
Water.....	.....	0.422
Acetic acid .....	0.8106	0.422
Tartaric acid .....	1.0331	0.322
Citric acid.....	0.8448	0.546
Sulphuric acid.....	0.6853	1.701
Hydrochloric acid.....	0.5037	1.949
Nitric acid.....	0.8445	1.960
Potassium acetate .....	1.3875	0.744
Potassium citrate.....	1.3066	0.843

These results are of importance in the estimation of tartaric acid as potassium hydrogen tartrate. Mineral acids should not be present nor any large excess of potassium acetate or citrate. On the other hand, solutions of potassium sulphate, nitrate and, especially, chloride have very little solvent action on the precipitated tartrate. Thus the solubility of the potassium hydrogen tartrate at 12° is 1 part in 3213 of a 5% solution of potassium chloride, and only 1 in 4401 of a 10% solution of the same salt.

Potassium hydrogen tartrate dissolves many oxides, forming double tartrates; tartar emetic is a compound of this character.

*Cream of tartar* consists chiefly of potassium hydrogen tartrate. Its composition and the mode of analysis are considered on page 712.

When potassium hydrogen tartrate is treated with solution of potassium carbonate or hydroxide until the liquid ceases to redden litmus paper, there results:

**Potassium tartrate;** neutral potassium tartrate. This forms colourless crystals freely soluble. When its solution is treated with an acid, the hydrogen tartrate is precipitated.

**Potassium sodium tartrate, Rochelle salt** is produced by neutralising cream of tartar with sodium hydroxide or sodium carbonate. It forms large crystals, containing 4 mol. of water, and is very readily soluble. Addition of acetic acid precipitates crystalline potassium hydrogen tartrate. This reaction distinguishes it from the sodium tartrate.

*Seidlitz powders* contain potassium sodium tartrate. Sometimes the tartrate is largely, and occasionally entirely, replaced by sodium hydrogen carbonate. Such a preparation would be strongly alkaline and notably different from Seidlitz powder. On the other hand, if the acid is in excess, the powder is apt to produce a turbid solution with water, owing to formation of potassium hydrogen tartrate.

In examining Seidlitz powders, the absence of notable proportions of sulphates should be proved, as a substitution of potassium hydrogen sulphate for tartaric acid is not unlikely. Some powders receive an addition of magnesium sulphate, or a minute quantity (0.01 gr.) of tartar emetic, whilst others are flavoured with lemon or ginger, and sweetened with sugar. Potassium chlorate is a constituent of some proprietary remedies of the nature of Seidlitz powders.

**Potassium Ferric Tartrate.**—It is prepared by adding precipitated ferric hydroxide to acid potassium tartrate and treating with cold water. It constitutes the *ferrum tartaratum* of pharmacy. The solution acidified with hydrochloric acid should give a copious blue precipitate with the ferrocyanides but none with the ferricyanides. It should yield 30% of  $\text{Fe}_2\text{O}_3$ , as estimated from the weight of the ash insoluble in water.

**Potassium Antimonyl Tartrate.**—**Tartarised antimony tartar emetic.** This is prepared by mixing antimonious oxide with potassium hydrogen tartrate, and subsequently adding water, boiling, filtering and crystallising. Cold water dissolves 7%, and boiling water 53% of the salt; the solution has an acid reaction. *Antimonial wine* is a solution of tartar emetic in wine.

Tartar emetic is now extensively employed for fixing certain coal-tar colours on cotton, its value for this purpose depending on the content of antimony. It is frequently largely adulterated, the percentage of antimony being sometimes scarcely one-half of that present in the pure substance.

The antimony may be conveniently estimated volumetrically, in a manner described by W. B. Hart (*J. Soc. Chem. Ind.*, 1884, 3, 294).

The sample is dissolved in water and sodium hydrogen carbonate added to the solution. Excess of a standard solution of calcium hypochlorite is then added. The excess is found by titrating back with a N/10 solution of sodium arsenite until a drop of the liquid ceases to give a blue with potassium iodide and starch. The strength of the hypochlorite solution is found by taking a measure equal to that added to the antimony solution and titrating with arsenite as before. 1 c.c. of a solution prepared with 4.95 grm. of pure arsenous oxide per litre has the same reducing power as 0.0060 grm. of antimony or 0.0072 of antimonous oxide.

**Potassium antimonyl oxalate** has been used as an adulterant of, and substitute for, tartar emetic. It is readily soluble, does not blacken on ignition or on heating with sulphuric acid, and gives a white precipitate on adding calcium chloride to the solution previously acidified with acetic acid. The salt yields only 23.7% of antimonous oxide.

**Ammonium tartrates** closely resemble the corresponding potassium salts, but are wholly volatile on ignition.

**Calcium tartrate** is a natural constituent of tartar from wine, the proportion contained being much increased if the wine has been "plastered." It also constitutes the greater part of the residue obtained on treating commercial tartars with hot water. Calcium tartrate is precipitated as a crystalline powder containing 4 mol. of water by adding excess of calcium chloride to a solution of a tartrate. It is soluble in 6265 parts of water at 15° and in 352 parts of boiling water. Strong acids and potassium hydrogen tartrate dissolve it readily; and hence it is frequently present in notable quantity even in purified tartars. These solutions are precipitated by ammonium hydroxide, either immediately or after some time. Calcium tartrate is soluble in ammonium chloride and in cold alkali, being reprecipitated from the latter solution on boiling. By digestion with a hot neutral solution of copper chloride it is converted into insoluble copper tartrate. This reaction distinguishes it from calcium *citrate*, but the reaction fails with mixtures containing a large proportion of *citrate*. The tartrate differs from the *racemate* and *oxalate* by its solubility in acetic acid. (For analyses of crude calcium tartrate see page 735.)

**Calcium racemate** is even less soluble in water than calcium tartrate, and is precipitated in fine needles on adding calcium sulphate

to a soluble racemate, or even to a solution of free racemic acid. Calcium racemate resembles the oxalate in being insoluble in acetic acid. It dissolves in hydrochloric acid to form a solution which is at once precipitated on adding ammonium hydroxide, whilst the tartrate is not precipitated for some time.

**Citric Acid.**—Citric acid occurs in a free state in the juices of many plants of the genus of *Citrus* (order, *Aurantiaceæ*), and also in the gooseberry, cranberry, currant, tamarind and many other fruits. The lemon, lime and bergamot are the fruits from which it is extracted. It has also been manufactured from unripe gooseberries, which yield about 1% of their weight of citric acid, besides containing malic acid. Good lemon-juice yields about 5.5% of crystallised citric acid. Calcium and potassium citrates are also widely distributed in the vegetable kingdom.

Citric acid is prepared from lime, lemon or bergamot juice, by neutralising the liquid with calcium carbonate, decomposing the resultant calcium citrate by an equivalent amount of sulphuric acid, and evaporating the liquid to the crystallising point.

Citric acid usually occurs as a crystalline powder or in transparent colourless prisms. In the trade, the crystals are assumed to have the composition  $C_6H_8O_7 + H_2O$ .

Crystallised citric acid begins to lose water at  $75^\circ$ , becomes anhydrous at  $135^\circ$ , fuses at  $153^\circ$ , and at about  $175^\circ$  decomposes into water and aconitic acid,  $C_6H_6O_6$ .

Citric acid has a strong acid taste, is soluble in about half its weight of water at  $25^\circ$  and 0.4 part of boiling water, in 1.5 parts of strong alcohol at  $25^\circ$  and 1.4 of boiling alcohol and in 18 parts of ether. The solution has no optical activity. Aqueous solutions readily become mouldy.

Citric acid is very soluble in dilute and absolute alcohol, but is nearly insoluble in ether, chloroform, benzene or petroleum spirit.

**Detection and Estimation of Citric Acid and Citrates.**—When 5 gm. of citric acid are heated with 30 c.c. of ammonium hydroxide for 6 hours in a sealed tube at a temperature of  $120^\circ$ , a yellow colouration is observed and small crystals are formed. If the cooled liquid be poured into an evaporating basin, it becomes blue in the course of some hours, the colour becoming more intense on standing, and in a few days turning to green, and ultimately disappearing. The change of colour goes on more slowly in the

dark. Heating the liquid on the water-bath hastens the production of the colour. Malic, tartaric, and oxalic acids do not interfere, even when present in large excess, but itaconic acid must be absent. It is said that 0.01 grm. of citric acid can be detected by this process (*Zeits. anal. Chem.*, 1878, **17**, 73).

Calcium citrate is very sparingly soluble, and less soluble in hot water than in cold. Hence, addition of excess of lime-water to a solution of citric acid produces but a slight precipitate in the cold, but a somewhat more considerable precipitate of calcium citrate is obtained on boiling, the deposit redissolving as the solution cools.

Precipitation as calcium citrate may be employed for the estimation of citric acid, and serves to separate citrates from *malates*, *acetates*, *formates* and *butyrates*; but the precipitate may contain calcium tartrate, oxalate or racemate.

Citric acid may be roughly separated from tartaric acid by digesting the mixed calcium salts with a hot and perfectly neutral solution of copper chloride, when soluble copper citrate is formed and an insoluble tartrate remains. In the case of mixed tartrates and citrates which can be converted into the calcium salts by precipitation with calcium chloride or nitrate in perfectly neutral boiling solution, this method of separation is occasionally convenient for qualitative purposes, but it is greatly inferior to the precipitation of the tartaric acid as potassium hydrogen tartrate, and fails wholly if the proportion of tartrate is small.

From *tartaric acid*, citric acid is best separated by the method described on page 712. In the filtrate from the precipitate of potassium hydrogen tartrate the citric acid may be determined by boiling off the alcohol, exactly neutralising with sodium hydroxide, and proceeding as directed on page 714, or by precipitation with barium acetate or lead acetate. If the acids do not exist in the free state, the solution must be prepared as directed under tartaric acid.

From *oxalic acid* citric acid is separated by neutralising the solution with sodium hydroxide, acidifying with acetic acid and adding calcium sulphate or chloride. After filtering from the precipitated calcium oxalate, the citric acid may be thrown down by adding lime-water and boiling.

If moderately pure, citric acid may sometimes be conveniently converted into barium citrate by precipitating the neutralised solution with barium acetate and adding 2 volumes of 95% alcohol.

After 24 hours the precipitate is filtered off, washed with alcohol of 63%, ignited, moistened with sulphuric acid, again ignited, and the weight multiplied by 0.601. Alkaline acetates do not interfere, so that the method is applicable to liquids from which the tartaric acid has been separated as potassium hydrogen tartrate.

In the absence of other acids, citric acid may be titrated with standard alkali, neutral litmus-paper being used. The alkali should be standardised with pure citric acid.

For the estimation of citric acid in presence of heavy metals, the latter should be first removed by hydrogen sulphide or sodium sulphids and the filtered liquid rendered neutral and precipitated with excess of lead acetate. The unfiltered liquid is mixed with an equal volume of alcohol, filtered, the precipitate washed with proof spirit and treated with ammonium hydroxide. The filtrate may contain citric and tartaric acids, but will be free from sulphates, phosphates and oxalates. When unmixed with other lead salts, lead citrate may be suspended in water, decomposed by hydrogen sulphide, the liquid filtered, well boiled and the citric acid in the solution titrated with alkali.

Full descriptions of the methods of determining citric acid in *juices* and *citric acid liquors* will be found in subsequent paragraphs.

**Commercial citric acid** frequently contains small quantities of *calcium salts*, due to imperfect manufacture, and traces of *iron*, *lead* and *copper* are also met with—these last being derived from the vessels used for the crystallisation and evaporation of the acid liquids.

The presence of all these impurities is indicated by igniting 5 or 10 grm. of the sample in a porcelain crucible. The ash usually ranges from 0.05 to 0.25%. When the ash does not exceed the latter amount, it is rarely of importance to examine it further, except for poisonous metals.

For the detection of lead the procedure is the same as with tartaric acid (page 716).

A colourless solution shows below

Faint blue solution shows below

Decided blue-yellow shows below

0.0003%.

0.0005%.

0.001%.

The presence of poisonous metals in citric acid is accidental, and the proportion present is usually small (1 part in 10,000); but as lead

and copper are occasionally present in dangerous amount, it is necessary to take every precaution to avoid their introduction.

If samples of citric acid contain *sulphuric acid*, they will be deliquescent. Sulphuric acid and sulphates may be detected and determined by acidifying rather strongly with hydrochloric acid and adding barium chloride. 233 parts of the precipitate correspond to 98 of sulphuric acid.

Formerly citric acid was liable to adulteration with tartaric acid. If present, tartaric acid may be conveniently detected by the charring which occurs on heating the sample with concentrated sulphuric acid, as described on page 712. When the proportion of tartaric acid in admixture with the citric acid is not too small, it may be detected by the dark mixture produced, within 5 minutes, when 1 grm. of the sample is dissolved in 10 c.c. of a cold saturated solution of potassium dichromate.

For the detection of tartaric acid in citric acid, Vulpius dissolves 0.5 grm. of the sample in 10 c.c. of distilled water, and adds 5 drops of the solution, drop by drop, to 15 c.c. of lime-water. If the citric acid contain mere traces of tartaric acid, a distinct turbidity will be produced in a few moments, which increases on adding more of the acid solution and stirring. In this manner 1% of tartaric acid may be detected.

If present in admixture with citric acid, *tartaric acid* is best estimated by the methods on pages 724 to 726.

**Citric Acid Liquors.**—This term is applied to the liquors resulting in citric acid works from the treatment of the calcium citrate with sulphuric acid. The assay is limited to the estimation of citric and sulphuric acids. For this purpose the total acidity may be determined by titration with standard alkali and phenolphthalein, and the sulphuric acid then determined. By subtracting the acidity due to the latter from the total found by titration, that due to the citric acid alone is ascertained. The free sulphuric acid is ascertained by treating 10 or 20 c.c. of the liquor with 5 times its volume of strong alcohol. After 11, and, if opalescence result, the whole is treated in the same way. The liquid is ultimately filtered, the precipitated sulphates dried with spirit and the filtrate precipitated with an alcoholic solution of calcium chloride. The precipitated calcium sulphate is allowed to settle completely, the supernatant liquor

poured off, and the precipitate and small quantity of remaining liquor *gently warmed*. The alcohol is gradually displaced by cautious additions of small quantities of water, and, when the precipitate has become crystalline, alcohol is added, and the precipitate collected on a filter, washed with alcohol, ignited, and weighed as calcium sulphate. The weight multiplied by 0.7206 gives the sulphuric acid ( $\text{H}_2\text{SO}_4$ ) in the liquor taken.

Another method, which agrees well with the above, is to neutralise exactly a known volume of the citric liquor with pure sodium hydroxide, evaporate to dryness, and ignite gently in platinum. The ash is wholly dissolved in a known quantity of standard acid, and the excess of acid ascertained by titration with alkali. (In presence of iron or aluminium, sodium tartrate or sodium potassium tartrate should be added before titration.) The acid neutralised by the ash is equivalent to the organic acid contained in the liquor used.

In old liquor, the citric acid should be precipitated as calcium salt, as other organic acids will be present in considerable amount. For this purpose the liquor is treated exactly as directed for juice.

**Lemon Juice; Bergamot Juice; Lime Juice.**—These juices contain citric acid; acids other than citric; citrates; salts of organic acids other than citric; salts of inorganic acids; and albuminous, mucilaginous, saccharine and indifferent substances. Alcohol is frequently added as a preservative, and mineral acids are not uncommonly employed as adulterants. Verjuice has also been used for the purpose.

J. Macagno finds that the alcoholic fermentation which takes place when freshly expressed lemon-juice is kept does not diminish the amount of citric acid present, but that this is succeeded by another fermentation which diminishes the citric acid, and other organic acids (chiefly acetic and propionic) increase. Similarly, juice expressed from rotten fruit contains acids other than citric, sometimes to the extent of 10%.

Citric acid juices lose some of their acidity by concentration. Warington observed a loss of 3.5% of the total free acid on concentrating English-pressed juice to one-sixth of its original volume. The loss is due, at least in part, to the presence of volatile organic acids, which, of course, exist in much smaller amount in concentrated juice. Warington found 1.25% of the total acidity of concentrated juice to be due to volatile acids. Among the latter were recognised formic, acetic and probably propionic acids.

The following table, compiled from Warington's data, shows the sp. gr., acid, and combined organic acid (the last two expressed in terms of crystallised citric acid) of the various citric juices commonly met with in commerce:

	Sp. gr.	Acid, oz. per gallon	Combined organic acid, oz. per gallon
Lemon juice:			
Raw Sicilian.....		6-9	0.85
Raw English.....	1.04 - 1.05	11-13	0.3
Concentrated.....	1.20 - 1.25	56-72	6-8
Bergamot juice:			
Concentrated.....	1.22 - 1.25	47-55	7-8
Lime juice:			
Raw.....	1.035-1.040	10.6-13.5	0.4-0.7
Concentrated.....	1.28 - 1.38	82-112	8-6

In the following table, due to Grosjean, are given estimations of the free acid and precipitable organic acid (both calculated as citric acid) in commercial samples of concentrated lemon and other juices:

	Sp. gr.	Acid (reckoned as citric acid), oz. per gallon		Proportion of pre- cipitable to 100 of acid
		Acid	Total acid precipitable	
Lemon juice:				
Average of 65 samples.....	1.241	62.1	61.6	99.2
Sample A.....	1.240	65.8	59.7	90.7
Sample B.....	1.235	64.9	55.7	85.8
Bergamot juice:				
Highest.....	1.235	47.9	48.5	101.4
Lowest.....	1.235	52.3	49.9	95.4
Lime juice:				
Sample A.....	1.326	108.3	99.8	92.2
Sample B.....	1.205	59.2	53.0	91.1
Orange juice:				
Sample A.....	1.400	16.8	11.6	69.0
Sample B.....	1.350	11.7	8.0	68.4

From the first of these tables it will be seen that English-pressed juice contains more free and less combined acid than the raw Italian

and Sicilian juices. This is probably due to the fact that the finest and ripest fruit is sent to England, whilst the windfalls and damaged fruit are treated locally.

Concentrated bergamot juice is far less acid than lemon juice, whilst concentrated lime juice is a thick viscid fluid far exceeding the others both in density and acidity.

**The analysis of genuine juice** is practically confined to the estimation of citric acid and citrates, and for this purpose the following processes are employed:

*Sp. Gr.*—A special hydrometer is sometimes used. On this "citrometer," 60 degrees correspond to a sp. gr. of 1.240, so that each degree appears to be equal to 0.004 sp. gr. above unity.

The valuation by sp. gr. is open to many frauds. Bergamot juice, which has a high gravity but low acidity, has been mixed with lemon juice, and sea-water has been added to the juice during concentration. Of course, the presence of alcohol materially affects the density, but it may be removed by boiling the juice and again taking the sp. gr. after making up the volume to that originally employed.

*Estimation of the Acid.*—This is effected by titration with N/2 sodium hydroxide, neutral litmus paper being used as an indicator. In the case of concentrated juice, 50 c.c. should be diluted to 500, and 25 to 30 c.c. of the diluted liquid employed for the titration. With unconcentrated juice, 10 or 20 c.c. may be measured out at once. In either case, the alkali is added in quantity sufficient to neutralise about 80% of the acid present; the liquid is then boiled for a few minutes, and when quite cold, the titration is completed. The neutralising power of the alkali should be known in terms of pure citric acid.

*Estimation of the Citrate and Other Organic Salts.*—This is effected by evaporating to dryness the portion of juice which has been already neutralised for the estimation of free acid. The residue left on evaporation is heated gradually, and charred at a low red heat. The ignited mass is treated with water, a known volume of standard sulphuric acid added, the liquid boiled and filtered, and the excess of acid ascertained in the filtrate by standard alkali. The amount of sulphuric acid neutralised by the ash is equivalent to the total organic acid of the sample, for on ignition all the salts of organic acids were converted into the corresponding carbonates. 49 parts of sulphuric acid neutralised = 40 of sodium hydroxide = 70 of  $\text{H}_3\text{C}_6\text{H}_5\text{O}_7, \text{H}_2\text{O}$ , or 67 of  $2\text{H}_3\text{C}_6\text{H}_5\text{O}_7, \text{H}_2\text{O}$ .

The result gives the total organic acid of the juice taken, calculated as citric acid. By subtracting the amount of free citric acid, obtained by titration of the acid juice, the amount of combined citric acid is ascertained.

If the original acid juice is evaporated and ignited, and the combined citric acid calculated from the neutralising power of the ash, the results obtained are too high, owing to the decompositions by the citric acid during evaporation.

*Estimation of the Real Citric Acid.*—Of the organic acids present in the genuine lemon and similar juices, the citric is the only one of importance which forms an approximately insoluble calcium salt. Calcium malate and aconitate are pretty freely soluble, and the same remark applies more strongly to calcium acetate and butyrate produced by the fermentation of citric acid juices. For the estimation of the amount of insoluble calcium salt obtainable from a citric juice, R. Warington recommends the following method (*J. Chem. Soc.*, 1875, **28**, 934): 15 to 20 c.c. of unconcentrated lemon juice, or about 3 c.c. of concentrated juice (previously diluted to facilitate exact measurement), should be exactly neutralised with pure sodium hydroxide. The solution is brought to a bulk of about 50 c.c. and heated to boiling in a salt or glycerin bath, and so much of a solution of calcium chloride added as is known to be rather more than equivalent to the total organic acids present. The whole is boiled for half an hour, and the precipitate then collected and washed with hot water. The filtrate and washings are concentrated to about 10 or 15 c.c., the solution being finally neutralised with a drop of ammonia if it has become acid. The second precipitate thus obtained is collected on a very small filter, the filtrate being employed to transfer it, and the washing with hot water being reduced as much as possible. In very accurate experiments the concentration should be repeated and any further precipitate collected. The precipitates, with the filters, are then burnt at a low red heat, and the neutralising power of the ash ascertained by treatment with standard hydrochloric acid and alkali. 1 c.c. of normal acid neutralised corresponds to 0.070 grm. of crystallised citric acid ( $C_6H_8O_7 \cdot H_2O$ ). The presence of mineral acids does not interfere; oxalic or tartaric acid would render the results inaccurate. It is desirable to add neutral hydrogen peroxide to the solution of the ash and boil before titration; otherwise an error may occur from the presence of sulphides.

**Citric Acid in Fruit Juices.**—The estimation as lead citrate, as described in *Bull.* 107, *Bur. of Chem., U. S. Dept. of Agr.*, has been abandoned as unreliable by the Association of Official Agricultural Chemists, and the following adopted in its place as a tentative method, applicable in the presence of sugar and malic and tartaric acids (*Arch. Chem. Mikros.*, 1914, 7, 285; *Abs. Z. Nahr.-Genussm.*, 1915, 30, 309):

Prepare the sample in exactly the same way as in their method II for the estimation of malic acid (page 708) up to the point where the precipitate of barium salts on the filter has been transferred back to the original flask with a jet of hot water. Boil until alcohol can no longer be detected by odour, and add enough sulphuric acid (1 to 5) to precipitate all the barium originally added and to allow 2 c.c. in excess. Evaporate by careful boiling to a volume of 60 to 70 c.c. and add 5 c.c. of freshly prepared saturated bromine water, or enough to show a distinct excess. Transfer with water to a 100 c.c. volumetric flask and dilute to the mark at standard temperature. Mix thoroughly, allow the precipitate to settle and filter through a dry filter. Pipette an aliquot part of the filtrate, containing not more than 400 mg. and preferably not less than 50 mg. of citric acid, calculated from the total acidity, into a 300 c.c. Erlenmeyer flask. Add 10 c.c. of sulphuric acid (1 to 1) and 5 c.c. of potassium or sodium bromide solution (15 gm. KBr in 40 c.c. or 16 gm. NaBr in 50 c.c. of water), mix, warm the flask in a water-bath to 48° to 50° and allow it to remain in the bath for 5 minutes. After removing from the bath, add rapidly from a burette 25 c.c. of potassium permanganate solution (5 gm. in 100 c.c. of solution), drop by drop with frequent interruptions, and with constant, vigorous shaking, avoiding a temperature during oxidation exceeding 55°. Set the flask aside until the hydrated peroxide of manganese begins to settle. The supernatant liquid should be dark brown, showing an excess of permanganate; if an excess is not indicated, add more. Shake again, set aside to settle and repeat this operation until the precipitate assumes a bright yellow colour and most of it has dissolved. Finally, while the solution is still warm, remove the last undissolved portion of hydrated manganese peroxide precipitate and also the excess of bromine by adding, drop by drop, clear ferrous sulphate solution (20 gm. of ferrous sulphate in 100 c.c. of water containing 1 c.c. of concentrated sulphuric acid). Allow the solu-

tion to cool, shaking occasionally. If the operations have been properly conducted, a heavy white precipitate of pentabromacetone is obtained which becomes crystalline on occasional shaking, and in this condition is entirely insoluble in water. Allow the mixture to stand overnight, collect it by means of gentle suction on a Gooch crucible provided with a thin pad of asbestos, previously dried over sulphuric acid in a vacuum desiccator, wash with water slightly acidified with sulphuric acid and finally wash twice with water. Dry the precipitate to constant weight over sulphuric acid in a vacuum desiccator, protecting it from strong light. The weight of pentabromacetone, multiplied by the factor 0.424, gives the equivalent weight of anhydrous citric acid ( $\text{H}_3\text{C}_6\text{H}_5\text{O}_7$ ). Occasionally the pentabromacetone is first obtained in the form of oily droplets. These become crystalline on standing or on cooling and are usually discoloured by negligible traces of manganese or iron.

This method may be applied directly to the sample without previous precipitation of the citric acid, as the barium salt when the amount of sugar or other permanganate reducing substances is not excessive. In this case begin the determination with the addition of 2 c.c. of sulphuric acid (1:5) and the treatment with bromine water.

A method for the analysis of *calcium citrate* and *lemon juice* has been recently described by L. and J. Gadais (*Bull. Soc. Chim.* [4], 1909, 5, 287): 20 grm. of the calcium citrate are boiled for a few moments with 30 c.c. of water and 20 c.c. of hydrochloric acid (sp. gr. 1.28), cooled, made up to 250 c.c., filtered through a dry filter, and 25 c.c. exactly neutralised with N/1 potassium hydroxide, using phenolphthalein, then treated with 1 c.c. of a saturated solution of calcium chloride, evaporated to 25 c.c., and filtered while very hot. The precipitate is washed 8 times with boiling water, using as little as possible, and dried at 105°. The filtrate is concentrated to 15 c.c., any additional precipitate washed 5 times with boiling water, sparingly as before, and dried at 105°. The filtrate and washings may be concentrated to 15 c.c., and any precipitate treated as with the other two. Finally, an equal volume of alcohol is added to the liquid, and a precipitate is added to the others, after drying. The collected precipitates are burnt apart from the filter, and the residue, calcium carbonate, mixed with 30 c.c. of N/1 hydrochloric acid, and the excess of acid ascertained by means of N/1 potassium hydroxide.

The number of cubic centimeters of acid required to neutralise the residue, multiplied by 0.07, gives the amount of citric acid that would be obtained from the sample. If the sample contains much sulphate, it is advisable to burn the precipitate over an alcohol flame, and to add to the residue 10 c.c. of hydrogen peroxide solution before adding the acid. (The fact that commercial hydrogen peroxide solution generally contains an appreciable amount of acid is not noted in the report of the process, but must not be overlooked. The peroxide solution should either be exactly neutralised or its acidity ascertained and allowance made.)

For *lemon juice*, 120 c.c. are diluted to 1000 c.c., 25 c.c. of this neutralised with N/1 potassium hydroxide, 20 c.c. of saturated calcium chloride solution added, and the procedure carried out as above.

In English-pressed lemon juice the real citric acid is 99% of the total organic acid, but in the concentrated Sicilian juice it ranges from 88 to 95% of the total. In a sample of concentrated bergamot juice, Warington found the precipitable acid to be about 88% of the total organic acid, but a more usual proportion is 96 to 98%. The method of determining the value of juice by its acidity usually, but not invariably, gives tolerably accurate results in the case of lemon and bergamot juice, but in lime juice the results are commonly in excess of the truth. Of course this statement is only true of genuine juice.

**Estimation of alcohol** can be effected by the usual methods.

**Adulterated lime and lemon juices** are not uncommon. The production of precipitates with barium chloride and silver nitrate indicates the presence of *sulphuric* and *hydrochloric acids*, respectively, pure juices containing merely insignificant traces of sulphates and chlorides. Free sulphuric acid may also be estimated as in citric acid liquors, and both that and free hydrochloric acid by *Hehner's* method for the determination of mineral acids in vinegar.

According to F. D. Scribani (*J. Chem. Soc.*, 1878, 34, 914), *nitric acid* has occasionally been used for the adulteration of lemon juice. On concentrating such juice the nitric acid decomposes the citric acid, either wholly or partially, with formation of oxalic, acetic, and carbonic acids; so that on neutralising the juice with lime a mixture of calcium salts is obtained. To detect the nitric acid, Scribani adds to the juice an aqueous solution of ferrous chloride, strongly acidu-

lated with pure hydrochloric acid and quite free from ferric salt. The liquid is then boiled for a few minutes and, after cooling, tested with a thiocyanate (sulphocyanide). If the liquid contain nitric acid, a more or less deep red colour will be produced, owing to the formation of a ferric salt. This test is said to answer equally well in presence of common salt or sulphuric or tartaric acid. In boiled and dark coloured juices dilution is necessary before the colour can be observed. A more satisfactory and direct test for nitric acid would be to boil the juice with metallic copper, when red fumes would be produced if nitric acid were present.

**Lime Juice, Lemon Juice, and Factory Citric Acid Liquors.**—The analysis of these materials is conducted as follows: 15 to 20 c.c. of unconcentrated juice, or an amount corresponding with 3 c.c. of concentrated juice (40 grm. per 100 c.c.), previously diluted to facilitate exact measurement, are exactly neutralised with pure potassium hydroxide (N/5). The liquid, having a volume of about 50 c.c., is heated to boiling, mixed with a slight excess of concentrated calcium chloride solution, and kept at gentle boiling for half an hour. The precipitate is filtered off immediately, while hot, washed with boiling water 6 times, and the mother liquor and washings again evaporated and worked up as described above under calcium citrate. The whole of the calcium citrate collected is then dissolved in 30 c.c. of N/1 hydrochloric acid and the excess of acid estimated as above. In dealing with the cruder factory liquors three or four evaporations are generally necessary to separate all the calcium citrate.

The above methods are not entirely free from error, (Cf. O. von Spindler, *Chem. Zeit.*, 1903, **27**, 1263), but have not yet been replaced by better. Incorrect results are obtained when the calcium citrate or juice contains other acids which yield sparingly soluble calcium salts. The presence of oxalic acid or of tartaric acid may be detected by the fact that the cold, neutralised solution gives a precipitate in the cold with calcium chloride.

**Other Methods of Estimation.**—The above-described methods are those which are generally adopted in the citric acid industry. The following methods, selected from the numerous processes which have been suggested, may be here briefly summarised.

C. Ulpiani and A. Parozzani (*Atti. R. Accad. Lincei*, 1906 [v], **15**, ii, 517) have described a method of analysis which, according to

Klapproth (*Lunge-Keane, Vol. III*), gives satisfactory results for citric acid, even in presence of other organic acids. This method depends upon the fact that citric acid, in presence of a sufficient quantity of calcium chloride, is precipitated by sodium hydroxide *in the cold*, when the whole of the acid is saturated, and in the *hot* solution when one-third of the acid is saturated.

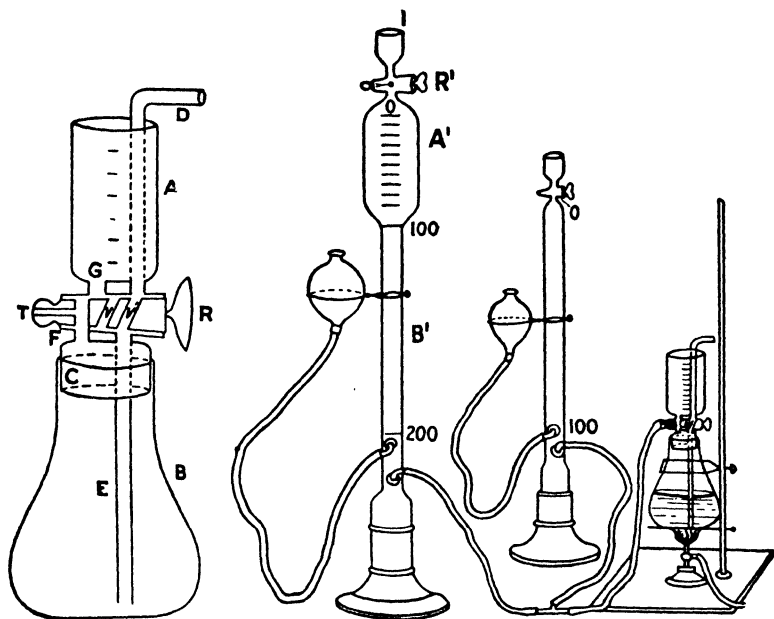


FIG. 105.

Spica (*Chem. Zeit.*, 1910, **34**, 1141; compare Barboni, *Ann. Lab. Centr. delle Gabelle*, 1912, p. 311) has described a method based on the formation of carbon monoxide when citric acid is gently warmed with concentrated sulphuric acid; 1 mol. of carbon monoxide is obtained for each molecule of citric acid present. The citric acid is first precipitated as calcium citrate (as in Warington's method) and the citrate then decomposed with the sulphuric acid in the apparatus described below (Fig. 105):

The upper part, *A*, is fitted to the flask, *B* (150 c.c. capacity), by a ground joint, and the tubes, *D* and *E* and *C* and *E*, may be connected respectively through the tap, *R*, as also may the flask and the

exterior. 2 gramm. of the calcium citrate, moistened with water, are introduced into *B*, and the air in the flask is completely displaced by carbon dioxide, the absence of air being ascertained by means of an auxiliary nitrometer, filled with potassium hydroxide solution (1:5) and attached to the T-piece. 25 c.c. of concentrated sulphuric acid are then run into *B* from *A*, and a slow current of carbon dioxide is again passed into the flask, which is warmed to 80° to 100°, and occasionally shaken, the carbon monoxide evolved being collected in a nitrometer of 200 c.c. capacity, of which the lower part *B*<sup>1</sup> (100 c.c. capacity), is graduated in fifths of a cubic centimeter. When the volume of gas becomes constant, the nitrometer is allowed to stand for half an hour and then, after washing the gas with potassium hydroxide solution, introduced through *I*, the volume is read and the usual corrections are made for temperature and pressure. 1 c.c. of carbon monoxide at 0° and 760 mm. indicates 0.009407 gramm. of citric acid ( $C_6H_8O_7 \cdot H_2O$ ). The same apparatus may be used for the estimation of carbonate in a citrate, by decomposing with a known volume of concentrated hydrochloric acid and measuring the evolved carbon dioxide over water.

The above process is obviously useless when other organic acids are present which evolve carbon monoxide.

**Estimation of Citric Acid in Presence of Other Acids.**—Beau's modification of the method of Denigès (*Ann. Chim. Phys.*, 1899, 18) is shown by Gowing-Scopes (*Analyst*, 1913, 38, 12) to give unreliable results owing to the action of the hydrogen peroxide (added to remove the manganese dioxide) upon the precipitated mercury dicarboxysulphoacetone. Other reagents tried for removing the manganese dioxide either oxidised or reduced the precipitate, which in hot solutions was also oxidised by manganese dioxide. A compound containing 72.5 to 74.0% of mercury and apparently similar to, if not identical with, the compound of Denigès was obtained by the use of a reagent prepared by adding 68 c.c. of strong nitric acid to 51 gramm. of mercuric nitrate and 51 gramm. of manganese nitrate, diluting the mixture with 100 c.c. of water, and finally, making up the solution to 250 c.c. and filtering. For the estimation of citric acid a quantity of the substance containing not more than 0.04 gramm. nor less than 0.001 gramm. of the acid is exactly neutralised with N/10 alkali, using phenolphthalein as indicator, and, after the addition

of 10 c.c. of the reagent, the liquid is diluted to 200 c.c., and boiled for 3 hours beneath a reflux condenser. The precipitate is washed by decantation, collected on a weighed Gooch crucible, and again washed in the crucible, which is then dried in the water-oven until nearly constant in weight (about 5 hours). The residue should then be of a cream colour, any yellow coloration indicating the formation of basic salts, which will cause the results to be too high. One-sixth of the weight of the precipitate gives the amount of citric acid. In 16 test estimations with pure citric acid within the limits stated above, the maximum error was  $+0.0003$  and  $-0.0004$  gram. Good results were also obtained in the presence of tartaric, succinic, oxalic, benzoic, and phosphoric acids, but when malic, lactic or salicylic acids were present the results were too high. Salicylic acid gives, with the reagent, a salmon-coloured precipitate, probably a nitro-derivative, whilst gallotannic acid gives an orange-brown precipitate. The precipitate given by citric acid decomposes suddenly when heated. It is very soluble in hydrochloric acid, in strong sulphuric or nitric acid, and in solutions of halogen salts.

In the absence of sugars it is not necessary to precipitate the citric acid, as barium citrate and redissolve with phosphoric acid. The author claims an accuracy of 2 to 4%.

The above process does not give satisfactory results in presence of malic and tartaric acid, and is therefore not applicable to the examination of fruit juices. In such cases, according to D. S. Pratt (*U. S. Dept. Agr., Bur. Chem. Circular 88, 1912*), the following method is of especial value:

50 gram. of fruit juice are treated with 110 c.c. of 95% alcohol to remove pectin substances. After 15 minutes the solution is filtered and the residue washed with 95% alcohol. The resulting solution is diluted with water to an alcoholic strength of about 50%, and a 20% aqueous solution of barium acetate is added to precipitate the citric acid. After stirring, allowing the precipitate to settle, and filtering the solution, the precipitate on the paper is washed with 50% alcohol to remove sugars, and then the paper and its contents are dried to remove the alcohol. The residue is next warmed with 50 c.c. of water and 3 to 5 c.c. of syrupy phosphoric acid to dissolve the barium citrate. This mixture is filtered into a graduated flask and the paper washed until the filtrate measures 100 c.c. An aliquot part of this solution, containing 0.05 to 0.15 gram. citric acid, is measured into a

500 c.c. distilling flask, 5 to 10 c.c. phosphoric acid are added with 400 c.c. of hot water, and the flask is heated. When briskly boiling 0.05% potassium permanganate solution is run in by means of a dropping funnel at the rate of 1 to 2 drops per second until the pink coloration is permanent.

The acetone formed by the oxidation distils off as fast as it is formed into 30 to 40 c.c. of Denigès<sup>1</sup> reagent. The distillation is continued till only 50 to 100 c.c. of solution remain in the flask.

The mixture in the receiver is boiled gently under a reflux condenser for 45 minutes after the liquid becomes cloudy. It is then filtered hot onto a Gooch crucible, washed with water, alcohol, and ether and dried in a water-oven for 30 minutes. The weight of precipitate multiplied by 0.22 gives the weight of citric acid originally present.

W. Fresenius and L. Grünhut (*Zeit. anal. Chem.*, 1913, 52, 31) claim that the methods based on the precipitation of acetone dicarboxylic acid, as given above, are untrustworthy in the qualitative detection of citric acid in wines. For this purpose they recommend Krug's modification of Moslinger's test, which is carried out as follows:

50 c.c. of wine are evaporated to a syrup. The syrup is treated with 95% alcohol and filtered to remove tartrates and then evaporated to remove alcohol. 10 c.c. of the resulting liquid are treated with acetic acid and lead acetate. In the presence of citric acid a precipitate will be obtained which dissolves on heating and reappears on cooling.

For more recent data as to the composition of grape juices and notes on the determination of their acidity, see Gore (*J. Ind. Eng. Chem.*, 1909, 1, July).

**Tests of purity of citric acid:** *Lead, arsenic, and ash* are tested for as under tartaric acid, the limiting quantities allowed being the same as in the case of tartaric acid (see page 716). It is usual, however, in commerce to require a higher degree of freedom from lead than in the case of tartaric acid; the amount present seldom exceeds 10 parts per million. Arsenic is generally entirely absent.

**British Pharmacopœia 1914.**—The following are the requirements: 1 gm. dissolved in water requires for neutralisation 14.2 c.c. of N/1

<sup>1</sup> Denigès reagent is made by adding 200 c.c. of concentrated sulphuric acid, with constant stirring, to a suspension of 50 gm. mercuric oxide in 500 c.c. of water. This mixture is heated on a water-bath to ensure complete solution. It is then filtered, cooled and diluted to 1000 c.c.

solution of sodium hydroxide; yields no characteristic reaction for copper or iron and not more than very slight reaction for calcium or sulphates. *Lead limit* 20 parts per million. *Arsenic limit* 1.4 parts per million. 1 grm. of powdered citric acid mixed with 10 c.c. of sulphuric acid in a test-tube previously rinsed with sulphuric acid acquires not more than a pale yellow colour when kept at a temperature of  $90^{\circ}$  for 1 hour (absence of tartaric acid); ash not more than 0.05%.

The following colour tests, based on Pusch's method of detecting tartaric acid in citric acid, are described by Hill (*Pharm. J.*, 1910, **84**, 245); 0.5 grm. of the sample and of pure citric acid are placed in separate test-tubes (6 in.  $\times$   $\frac{5}{8}$  in.) and 5 c.c. of sulphuric acid added to each. The tubes are placed simultaneously in an ordinary Bunsen flame; at the end of 30 seconds they are withdrawn and examined. The results obtained with acids of different degrees of purity are as follows:

Pure acid	= lemon-yellow solution.
5.0% of tartaric acid	= black, sulphur dioxide abundant.
1.0% tartaric acid	= deep brown-black, sulphur dioxide evident.
0.5% tartaric acid	= deep red-brown.
0.25% tartaric acid	= red-brown.
0.1% tartaric acid	= reddish-brown.
0.01% tartaric acid	= brownish-yellow.

With careful manipulation and 60 seconds' heating, 0.0001% of tartaric acid is said to be easily detected; even 0.00001% gave a distinctly deeper tint, in good light, than the control tube. The test is also applicable to citrates, 1% of tartrate being easily detected. The above method is also applicable to detect sugar in citric acid; the reactions obtained are:

1% of sugar	= cherry-red, sulphur dioxide distinct.
0.1% of sugar	= cherry colour, sulphur dioxide evident.
0.01% of sugar	= yellowish-red, sulphur dioxide perceptible.
0.001% of sugar	= reddish-yellow.
0.0001% of sugar	= reddish-yellow.

It was also found that 0.5% of sugar could be detected, in tartaric acid, by the coloration after an hour's contact with cold sulphuric acid; by heating for 10 seconds in the flame the control tube remained

practically unaffected; with 1% of sugar a red solution was obtained; and with 0.5% a red-brown ring.

Häussler (*Chem. Zeit.*, 1914, 38, 937) describes a characteristic colour reaction given by citric acid with vanillin; the solution is evaporated to dryness after adding an alcoholic solution of vanillin and the residue treated with 3 drops of dilute sulphuric acid, heated on a water-bath for 15 minutes, dissolved in water and ammonia added. A bright-red coloration is obtained with 0.002 grm. of citric acid. The red coloration is not given by tartaric, malic, xalic, maloonic, benzosalicic, ylic, acetic, lactic or succinic acids.

**Citrates.**—Citric acid forms three classes of salts. It has a great tendency to produce stable double citrates, and hence many metallic solutions are not precipitable by alkalis in presence of sufficient citric acid. This fact is often utilised in analysis.

No metallic citrate is wholly insoluble in water. Calcium citrate is one of the least soluble, and hence is employed in the estimation of citric acid. General reactions of the citrates are described elsewhere, and the properties of the more important commercial forms are given below.

**Lithium Citrate.**—As usually prepared, this is a white powder, but it may be obtained in crystals with 4 mol. of water. The salt is generally stated to be deliquescent, but this is an error. It should be soluble without residue in 25 parts of cold water.

The pure salt, after being rendered anhydrous by drying at 115°, on ignition leaves 52.9% of lithium carbonate. The residue should be treated with ammonium carbonate, and again ignited very gently, as it is liable to lose carbonic acid. A higher ash than the above indicates impurity or adulteration by (probably) *sodium citrate*, which leaves 61.5% on ignition. 1 grm. of anhydrous lithium citrate leaves on ignition a residue which should neutralise at least 14 c.c. of normal hydrochloric acid. The same amount of sodium citrate (after ignition) would only neutralise 11.25 c.c. of acid. If the resultant solution be evaporated to dryness, lithium chloride may be dissolved out of the residue by a mixture of equal volumes of alcohol and ether, while any potassium or sodium chloride will remain undissolved.

Much of the commercial lithium citrate contains *lithium carbonate*. This gives it an alkaline reaction and increases its ash and saturating capacity. Excess of citric acid gives the salt an acid reaction and

reduces the percentage of ash and saturating power. Hence, these impurities can be distinguished from sodium citrate, which *raises* the ash and *diminishes* the saturating power of the sample.

**Potassium salts** may be detected by adding tartaric acid to the concentrated solution of the sample and stirring, when a white crystalline precipitate of acid potassium tartrate will be produced.

**Insoluble matters**, such as powdered petalite or lepidolite, will be left undissolved on dissolving the sample in hot water, and *calcium* compounds may be estimated in the solution by adding ammonium oxalate.

**Calcium Citrate.**—This is a white substance, very sparingly soluble in cold, and still less in hot water. It is produced, in an impure state, by the citric acid manufacturer by boiling the juice with calcium carbonate, and is offered in the market as a convenient source of citric acid. The product consists essentially of citrate mixed with other salts of calcium, and excess of lime or calcium carbonate. In Sicily, dolomitic lime is sometimes used for neutralising the juice, in which case magnesium salts will be present. It is particularly liable to decompose if the percentage of moisture is considerable (more than 10 or 12%), and therefore some samples contain scarcely any real citrate.

The analysis of commercial calcium citrate is now generally carried out by Warington's method modified as follows:

4 grm. of the citrate are boiled with 30 c.c. of N/2 hydrochloric acid, in a 100 c.c. standard measuring flask for 10 minutes, the solution being then cooled and made up to the mark with water. It is then shaken and filtered through a dry filter paper, 50 c.c. of the filtrate being measured by a standard pipette into a beaker of 300 c.c. capacity and exactly neutralised with dilute sodium hydroxide free from carbonate, using phenolphthalein as indicator. The solution is next made slightly acid by the addition of 3 or 4 drops of N/1 hydrochloric acid, 2 c.c. of a 45% solution of calcium chloride added, the liquid raised to the boiling point and kept boiling for 15 minutes; to avoid bumping it is necessary to stir the liquid well until actually boiling, after which it can safely be left. The hot liquid is filtered, and the precipitate on the filter paper washed with boiling water 6 times. The filtrate and washings are then made just alkaline by adding a drop or two of dilute ammonia, and boiled down to about 15 c.c. The precipitate which forms is collected on a small

filter paper and washed with *boiling* water 6 times, a very small quantity of water being used for each washing. The filtrate and washings are treated with a drop of ammonia, if they have become acid, and are boiled down to about 10 c.c., but as a rule no further precipitate will be obtained whilst the liquid is hot; any precipitate which forms on cooling can be neglected.

The filter papers with their precipitates are dried at  $100^{\circ}$  and burnt together in a platinum dish with a cover. The flame should be kept low until the whole is charred, and then gradually raised until the ash is white. The mass is then carefully treated with 30 c.c. of N/1 hydrochloric acid, and boiled until all is dissolved and all carbon dioxide expelled; the resulting solution is titrated with N/5 or N/2 sodium hydroxide, using phenolphthalein as indicator.

The sodium hydroxide is standardised with pure potassium hydrogen tartate, and the N/1 hydrochloric acid with the alkali; phenolphthalein is used as indicator.

The number of cubic centimeters of N/1 HCl used for the neutralisation of the ash  $\times 0.070$  gives the weight of citric acid in the portion tested.

An almost identical method has been described by L. and J. Gadais (*Bull. Soc. Chim.*, 1909 [iv], 5, 287).

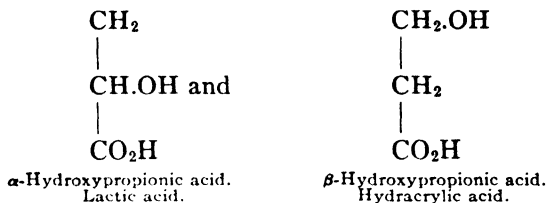
If the citrate contains much sulphate, it is advisable to ash at as low a temperature as possible, preferably with an alcohol flame. Before dissolving in hydrochloric acid, the ash should be treated with 10 c.c. of hydrogen peroxide. (If, as is usual, the hydrogen peroxide contains free acid, allowance must, of course, be made for it.)

**Ferric citrate** may be obtained by dissolving ferric hydroxide in citric acid and evaporating the solution in thin layers. It is thus obtained in transparent garnet-red scales, which are permanent in the air. It is insoluble in alcohol, but dissolves slowly in water to form a solution of a faintly ferruginous taste, not precipitated by ammonium hydroxide, but yielding ferric hydroxide on boiling with sodium hydroxide. After drying at  $100^{\circ}$ , the scales should leave from 29 to 30% of residue on ignition.

Iron ammonium citrate may be made by dissolving precipitated ferric hydroxide in a solution of citric acid and adding ammonia. It occurs in thin, transparent, deep red scales, slightly sweetish and astringent. When heated with potassium hydroxide its solution evolves ammonium hydroxide and deposits ferric hydroxide. The

alkaline liquid filtered from the precipitate should not give any crystalline precipitate or streaks of potassium hydrogen *tartrate*, when acidulated with acetic acid and vigorously stirred. Ferric ammonium citrate is readily soluble in water, forming a faintly acid solution, but is almost insoluble in 95% alcohol.

**Lactic Acid.**—Two chemically isomeric forms of hydroxypropionic acid exist having the structures



$\alpha$ -Hydroxypropionic acid, moreover, by virtue of its containing an asymmetric carbon atom (indicated in *italics*) exists in three optically different forms, viz., dextro-lactic acid (sarcosolactic acid), lævo-lactic acid, and inactive or ordinary (fermentation) lactic acid. Of these substances, the last is by far the most important.

**Inactive Lactic Acid,  $\text{C}_3\text{H}_6\text{O}_3$  or  $\text{CH}_3.\text{CHOH}.\text{CO}_2\text{H}$**

Lactic acid was first obtained by Scheele, in 1780, from sour milk. It exists ready formed in both the animal and the vegetable kingdoms. Lactic acid is formed by the lactic acid fermentation of carbohydrates such as sugar, gum, starch, mannitol, and particularly of milk-sugar, in the presence of casein or other proteins. Hence lactic acid is contained in sour milk (but not in fresh milk), in sauerkraut, pickles, distillery-wash,<sup>1</sup> sour beer, etc. The acid contained in sour tan-liquors and the acid runnings of starch-makers, etc., called by Braconnot nanceic acid, and the so-called thebolactic acid contained in opium are also ordinary lactic acid. Lactic acid is present in molasses (Schöne and Tollens, *Zeit. Ver. deutsch. Zuck. Ind.*, 1900, 980; Weisberg, *Bull. Assoc. Chim. de Suc.*, 1901, 18, 545).

Lactic acid is obtainable by various synthetical processes, including: the action of nitrous acid on alanine; the oxidation of

<sup>1</sup> Spent distillery-wash, made from grain, technically called slop or slop ale contains about 3% of solid matters of which about 1% consists of lactic and other acids, 0.7 of peptones and other nitrogenous matters, and 0.7% mineral matters, in which phosphates predominate. When made from molasses, the solids are less in amount and the ash is composed largely of potassium sulphate and carbonate.

$\alpha$ -propyleneglycol by nitric acid; the action of alkalis on  $\alpha$ -chloro- or bromopropionic acid; the reaction of aldehyde with hydrocyanic acid, and treatment of the resulting hydroxycyanide with hydrogen chloride; the cautious oxidation of glycol with spongy platinum or dilute nitric acid; the reduction of pyruvic acid,  $\text{CH}_3\text{CO.CO}_2\text{H}$ , etc.

It is also formed in considerable quantity by the action of alkalis on certain carbohydrates under suitable conditions, more particularly in absence of air or oxidising substances (see Kiliani, *Ber.*, 1882, 15, 699). Thus invert sugar yields with alkalis 50 to 60% of lactic acid (together with 0.5 to 2% of formic acid and 40 to 50% of polybasic acids). Galactose yields only 20% of lactic acid (Meisenheimer, *Ber.*, 1908, 41, 1010).

Lactic acid is a regular constituent of wine and at one time it was thought that it was an intermediate product in the alcoholic fermentation of sugar. But the fact that the acid is not fermentable by yeast (Slator, *Trans.*, 1906, 89, 141; 1908, 93, 231; Buchner and Meisenheimer, *Ber.*, 1910, 43, 1773), makes the hypothesis improbable. Its presence in wine is apparently to be attributed to the fermentation of malic acid by the *Micrococcus malolacticus* (Seifert, *Chem. Centr.*, 1907, ii, 346; Rosenstiehl, *Compt. rend.*, 1908, 147, 150).

Lactic acid is commercially prepared by the lactic fermentation of sugar (for details see Claflin, *J. Soc. Chem. Ind.*, 1897, 16, 516; Shafer, *Chem. Zeit.*, 1907, 6, 177 and 189; McLauchlan, *Int. Congress Appl. Chem.*, 1909, Section IV a, 1, 141).

The formation of lactic acid by the fermentation of glucose may be represented by the equation  $\text{C}_6\text{H}_{12}\text{O}_6 = 2\text{C}_3\text{H}_6\text{O}_3$ , but the action is much less simple than is thus indicated. Numerous organisms are capable of converting sugar into lactic acid, but that known as the lactic ferment (*Bacillus acidi lactici*), especially the Bulgarian variety, has by far the most energetic action. It consists of short thick cells generally united in pairs, and is most active between 35° and 45°. Access of air is necessary, and nitrogenous food is required. Excess of acid arrests the fermentation, but the action recommences if the liquid be neutralised. The action is not affected by 2% of alcohol, but 4% diminishes it, and 6% arrests it completely.

To prepare lactic acid in the laboratory the following method is given by McLauchlan. It is similar in principle to the method followed on the large scale: 400 grm. of commercial glucose (70%

grape sugar) is dissolved in 1200 c.c. of hot water, 170 grm. of powdered calcium carbonate added and 20 grm. of malt which has previously been boiled a few minutes with 100 c.c. of water. The mixture is then kept gently boiling during 30 minutes in order to sterilise the raw material, cooled to 60° to 70° and after being well stirred, is poured into a narrow-mouthed bottle of a capacity of 2 to 2.5 litres. Any calcium carbonate is rinsed out with water until the volume of liquid amounts to 1800 c.c. The bottle is then stoppered with a wad of cotton wool and placed in an incubator maintained at a constant temperature of 45°. The fermentation is started by means of a pure culture of the lactic ferment, which may be prepared as follows: Sweet milk is sterilised in a number of test-tubes, which are then closed with a plug of cotton-wool and placed in the incubator. Tube No. 1 is inoculated with a drop of sour milk, and 1 day later tube No. 2 inoculated from tube No. 1; then, on the next day, tube No. 3 is inoculated from No. 2 and the process repeated until a pure culture is obtained in the seventh tube.

A few grams of the pure culture are added to the sugar mixture and, after a few hours, the progress of the fermentation becomes visible by the evolution of carbon dioxide when the bottle is shaken. The bottle should be shaken every 6 hours or so, and after a week a sample should be tested daily, by means of Fehling's solution, for the percentage of sugar remaining in solution. When this falls below 0.4% the fermentation is finished. The calcium lactate is poured into a large beaker and sulphuric acid equivalent to the calcium present added. After thorough stirring, a test-tube full is filtered and tested with a drop of sulphuric acid to ascertain whether sufficient acid has been added to precipitate all the calcium as calcium sulphate. When this point has been reached the calcium sulphate is removed by filtration on the pump, and the solution concentrated, preferably *in vacuo*, when more calcium sulphate separates and is removed by filtration through charcoal which decolorises the liquid. The final filtrate should amount to 1 litre of 25% lactic acid.

A purer lactic acid can be obtained by cooling the solution obtained by fermentation so as to obtain crystals of calcium lactate which is then recrystallised and the product decomposed by sulphuric acid.

*Commercial Lactic Acid.*—In America lactic acid is generally sold in the form of solution with an acidity representing 22 or 44% of

lactic acid. A solution with a concentration of 50% is widely used in England and Germany. For lactic acid conforming to requirements of the British and U. S. Pharmacopœia see page 780.

*Pure anhydrous lactic acid*, which is obtained by repeated fractionation of the concentrated liquor in a vacuum of 0.5 to 1 mm., forms a crystalline but very hygroscopic mass which melts at 18° and boils at 82° to 85° under the pressure given and 119° to 120° under 12 mm.

Lactic anhydride and lactide are formed when lactic acid is evaporated at the ordinary temperature in dry air. The following table shows the percentage composition of products thus obtained by Wislicenus:

	Water	Lactic acid, $C_3H_5O_3$	Lactic anhydride, $C_6H_8O_4$	Lactide, $C_6H_8O_4$
A. Freshly prepared as above; syrup	15.64	58.80	25.56	
B. After drying 4 months over sulphuric acid	4.07	22.43	73.50	
C. After 13 months; thick syrup insoluble in water.			97.85	2.06
D. After 16 months; molasses-like syrup			71.41	28.69
E. After 18 months; thick gummy mass			60.77	39.50

Lactic acid is not volatile under ordinary pressures without decomposition. At 130° it begins to decompose, and at about 145° sparingly soluble lactic anhydride is formed, which at a higher temperature forms lactide and other products.

Lactic anhydride and lactide are nearly insoluble in water, but are converted by prolonged boiling with water, and readily by solutions of alkali hydroxides into lactic acid. The lactide obtained by heating paralactic acid (dextrorotatory) yields ordinary inactive lactic acid when treated in this manner.

Lactic acid is miscible in all proportions with water, alcohol, glycerol, and ether. It is but slightly soluble in chloroform, and is insoluble in carbon disulphide and petroleum spirit. Glyceric acid, which resembles lactic acid, is insoluble in ether.

Lactic acid dissolves freshly precipitated calcium phosphate, and is frequently used for that purpose.

Concentrated sulphuric acid mixes with pure lactic acid without blackening it. On heating, a brown colour is developed, and much carbon monoxide evolved, a humus-like substance being ultimately left. When heated with dilute sulphuric acid a mixture of acetaldehyde and formic acid is obtained.

On distillation with a large excess of quicklime, lactic acid is converted into carbon dioxide and alcohol.

Lactic acid does not reduce Fehling's solution, but rapidly decolorises potassium permanganate, both in acid and in alkaline solutions, with production of an odour of aldehyde. Silver lactate is imperfectly reduced on boiling, with production of a blue liquid and a brownish deposit.

Lactic acid may be separated from organic acids forming insoluble lead salts by precipitating the solution (previously neutralised if necessary) with neutral lead acetate, either with or without an addition of alcohol. Lead lactate remains in solution and may be decomposed with hydrogen sulphide, when free lactic acid is obtained.

Many admixtures may be separated from lactic acid by saturating the free acid with barium carbonate. When the aqueous solution is evaporated and the residue treated with alcohol, many of the acids whose barium salts are soluble in water remain behind, whereas barium lactate dissolves in alcohol. Free lactic acid may be obtained by cautiously precipitating the solution of barium lactate with dilute sulphuric acid, and filtering.

When purified from all substances except those soluble in alcohol, the aqueous liquid containing free lactic acid may be saturated with zinc oxide, evaporated to dryness, and the residue digested with alcohol. Zinc lactate, insoluble in alcohol, remains, while the other matters dissolve. After drying at  $120^{\circ}$ , the residue may be weighed, and its weight, multiplied by 0.7400, gives that of the lactic acid. Zinc sarcosylactate dissolves readily in alcohol, so the above process is useless for the estimation of sarcosylactic acid. With inactive lactic acid it yields fairly approximate results, with careful manipulation and under favourable circumstances.

According to R. Palm (*Zeit. anal. Chem.*, 16, 33), when treated with lead acetate and alcoholic ammonia, lactic acid is completely thrown down as a heavy granular precipitate of the formula  $3\text{PbO}$ ,  $2\text{C}_3\text{H}_6\text{O}_3$ . To examine an animal or vegetable organ for free lactic acid, Palm extracts it with ether (previously acidifying with sulphuric acid if a lactate is under treatment), evaporates the ethereal solution to a syrup, and treats the residue with water. The filtered aqueous solution is mixed with lead acetate, and any precipitate produced is filtered off. On adding more lead acetate to the filtrate, followed by alcoholic ammonia, the lactic acid is thrown down free

from foreign substances. The precipitate may be washed with alcohol, in which it is quite insoluble, and the contained lactic acid estimated from the loss on ignition. Minute traces of lactic acid may be thrown down with greater certainty by shaking the filtrate from the first lead precipitate with an excess of freshly precipitated lead hydroxide. In either case the precipitate is stated to yield pure lactic acid when decomposed with hydrogen sulphide and extracted with ether.

Calcium lactate is rapidly fermented by certain bacteria, giving rise to volatile fatty acids; in some cases butyric acid is the principal product, in others propionic and *n*-butyric acid predominate.

**Qualitative Tests.**—Windisch (*Chem. Centr.*, 1887, 826) proposed a method of detecting small quantities of lactic acid by treating the substance with chromic acid, whereby formic acid and aldehyde are produced. The solution to be tested is diluted to about 100 c.c., 5 c.c. of concentrated sulphuric acid and a little potassium dichromate added, and the liquid distilled. The vapours are received in warm Nessler's solution, with which, in presence of aldehyde, lead salts give a yellowish-red precipitate, or, with smaller quantities, a yellowish opalescence. Formic, acetic, propionic, butyric, valeric, succinic, malic, citric, and tartaric acids are said not to give the reaction, but alcohol, ammonia, and sugar must be absent. Small quantities of lactic acid are liable to escape detection by this method, owing to the oxidation of aldehyde to acetic acid by excess of chromic acid. To examine roots for lactic acid, they are first exhausted with ether, which is said to extract all substances of an acid nature.

A sensitive test for lactic acid is given by Denigès (*Bull. Soc. Chim.*, 1909, [iv], 5, 647), which is based on the formation of acetaldehyde on heating lactic acid with sulphuric acid. 0.2 c.c. of a solution of lactic acid (up to 2% strength) is heated with 2 c.c. of sulphuric acid (sp. gr. 1.84) at 100°, during 2 minutes. To the cold mixture a drop of an alcoholic solution of guaiacol or codeine is added; with the former a rose-red tint is produced and with the latter an orange-red coloration. This test serves to distinguish lactic acid from glycollic acid, which gives rise under the conditions named to formaldehyde, and hence produces different colorations from those shown by lactic acid.

A test due to Vournasos (*Zeit. angew. Chem.*, 1902, 15, 172) and modified by Croner and Cronheim (*Berl. klin. Wochenschr.*, 1905,

42, 1080) for lactic acid is based on its conversion into iodoform and of the latter into phenyl isonitrile, which can be recognised when present in very small quantity by its smell. 2 grm. of potassium iodide are dissolved in water, 1 grm. of sublimed iodine added, and the solution filtered through glass wool or asbestos, and made up to 50 c.c. A few cubic centimeters of the liquid to be tested are made alkaline with 10% potassium hydroxide solution, after which the liquid is boiled for a few minutes and then mixed with the aniline-iodine solution. The presence of lactic acid is shown by the well-known isonitrile odour being detected. Care must of course be taken that no other substances (*e. g.*, alcohol, acetone, etc.), which are capable of giving rise to iodoform under the conditions named, are present. If such substances are present, the test is valueless.

According to Thoms (*Zeit. physiol. Chem.*, 1907, 50, 540) the following test is preferable, as it is not shown by butyric acid, acetic acid, acetone, alcohol or hydrochloric acid. The gastric extract is heated with a few drops of 30% chromic acid for 10 minutes on the water-bath, when a reddish-brown colour is given by traces of lactic acid.

Fletcher and Gowland Hopkins (*J. Physiol.*, 1907, 35, 247) recommend the following test: 5 c.c. of sulphuric acid, a drop of a saturated solution of calcium sulphate and a few drops of the suspected solution are heated in a test-tube placed in a water-bath for 2 minutes. The tube is cooled and 2 to 3 drops of a dilute solution of thiophen (10 to 50 drops in 100 c.c. of alcohol) added. On again heating in the water-bath, the fluid becomes light cherry-red in colour if lactic acid is present. This is due to an aldehyde reaction with thiophen.

For the detection of free lactic acid in gastric juice, Uffelmann (*Zeit. klin. Med.*, 7, 392) prepared a reagent by mixing 10 c.c. of a 4% solution of phenol with 20 c.c. of water, and adding 1 drop of solution of ferric chloride, British Pharmacopœia. This forms a clear liquid of an amethyst colour, which is turned yellow by a solution of lactic acid containing only 1 part in 10,000. Hydrochloric acid in small quantities has but little effect, and when present in large amount simply decolorises the reagent. The test is preferably applied to the ethereal extract obtained as described on page 771, as in that case the traces of thiocyanates normally present in the stomach do not interfere.

This test is not, however, characteristic of lactic acid, but is responded to by tartaric, citric, malic and oxalic acids (Kühl, *Milchw. Zentr.*, 1910, **6**, 61). It gives negative results with benzoic and lævulinic acids. If used for the detection of this group of acids, it may be improved by substituting a 1/400% solution of salicylic acid for the phenol. Benzoic acid may also be used as follows: 5 c.c. of a 0.002% solution are mixed with a drop of ferric chloride (1:10) when a white turbidity with a green reflex is formed. On adding a few drops of a weak solution (0.001%) of lactic, tartaric, citric, malic or lævulinic acid, the liquid becomes clear and turns a dark greenish colour. The reaction is also given by ammonium oxalate.

**Separation of Lactic Acid from Other Organic Acids.**—According to Schoorl (*Zeit. angew. Chem.*, 1900, **15**, 367) succinic, malic and lactic acids are separated from oxalic, tartaric and citric acids by adding excess of milk of lime, boiling and filtering. After evaporating to dryness, 70% alcohol extracts calcium lactate, with traces of benzoate and salicylate, if present. The residue contains malate and succinate. Lactic acid is oxidised to acetaldehyde by potassium permanganate and dilute sulphuric acid (malic acid also gives acetaldehyde). By dissolving calcium lactate in dilute acetic acid and adding cobalt or zinc acetate, the corresponding lactate is obtained by slow evaporation in the air and recognised by its crystalline form. Formic, acetic, benzoic and salicylic acids are volatile with steam, and are thus easily separated from the less volatile lactic acid; it must be remembered, however, that lactic acid is slightly volatile with steam (Müller, *Bull. Soc. Chim.*, 1896, **15**, 1206. Partheil, *Zeit. Nahr. Genussm.*, 1902, **5**, 1053. Utz, *Chem. Zeit.*, 1905, **29**, 363).

According to Schneider (*Zeit. anal. Chem.*, 1899, **38**, 775) lactic, butyric and valeric acids are separated by distilling with superheated steam, when butyric and valeric acids pass over leaving lactic acid in the residue. To identify the lactic acid the contents of the retort are evaporated with zinc oxide and filtered whilst hot. Zinc lactate crystallises on cooling. A few centigrams of the zinc lactate are mixed with phosphoric acid and extracted with ether. The ether is evaporated and a little cobalt acetate and lead acetate added; a precipitate is obtained of cobalt lead lactate in the form of colourless, hexagonal plates.

**Estimation of Lactic Acid.**—Numerous methods have been described for this purpose, but none seems entirely free from objection. In most cases the difficulty arises from the presence of other substances which are liable to be returned as lactic acid owing to their behaving in the same manner as this substance in the conditions of the experiment. The method of Chapman and Smith, for example, described in the third edition of this work, gives unreliable results because of the presence of other easily oxidisable substances.

*Buchner and Meisenheimer's Method.*—According to Buchner and Meisenheimer (*Ber.*, 1908, **41**, 1416 and 1910, **43**, 1784) the most accurate method of separating and estimating lactic acid when present with other substances is as follows:

10 c.c. of the solution (or an amount of material containing about 1 grm. of lactic acid) is mixed with 10 c.c. of dilute sulphuric acid and 10 c.c. of saturated sodium sulphate solution and extracted continuously during 72 hours with ether in a von der Heide extraction apparatus (*Ber. K. Lehranstalt f. Weinbau in Giesenheim*, 1906, 253). The ethereal extract is dried during 18 hours with anhydrous sodium sulphate, the solution filtered from the sodium sulphate and the latter thoroughly washed with ether. The ethereal filtrate is evaporated to a thick syrup (to remove volatile acid), which is taken up with water and boiled with zinc carbonate. After filtering and thoroughly washing the residual zinc carbonate with water, the solution is evaporated to 5 c.c., 15 c.c. of alcohol added, and the solution left to crystallise overnight. The crystals are then collected on a Gooch crucible, washed with 75% alcohol, and dried at 105°. The result is calculated from the relation  $\text{Zn}(\text{C}_3\text{H}_5\text{O}_3)_2 = 2\text{C}_3\text{H}_6\text{O}_3$ , and is apparently 5 to 10% low. Thus 0.985 grm. of pure lactic acid taken gave a result 0.934.

If non-volatile acids which form zinc salts that are sparingly soluble in dilute alcohol be present, the method is useless.

If in the above method formic acid be present, there is danger of zinc formate crystallising from the alcoholic solution with the zinc lactate, unless the formic acid has been completely expelled during the evaporation of the ethereal solution to a syrupy consistence. On the other hand, if the heating of this syrup be continued too long there is danger of loss of lactic acid by volatilisation; this is probably the reason of the low results recorded. If volatile acids or alcohols be

removed by steam distillation prior to the ether extraction, there is also danger of loss of lactic acid.

The above method has the advantage that from the proportion of water of crystallisation and of zinc oxide in the zinc lactate obtained it can be ascertained whether the lactic acid is the optically inactive or active form (inactive =  $3\text{H}_2\text{O}$ ; active =  $2\text{H}_2\text{O}$ ). The polarimeter can be used to distinguish between the *d*- and *l*-forms.

*Kunz' Method.*—This method (*Zeit. Nahr. Genussm.*, 1901, 4, 673) was devised for estimating lactic acid in wine. For this purpose 20 c.c. of wine are mixed with a slight excess of powdered barium hydroxide and evaporated to about two-thirds of the original volume. When cold, the whole is rinsed into a 200 c.c. flask, made up to the mark, and filtered; 150 c.c. of the filtrate are evaporated to a thin syrup after neutralising the excess of baryta with carbon dioxide, and, when cold, excess of dilute sulphuric acid is added, and the whole introduced into a Schacherl extraction apparatus and extracted with ether during 18 hours [Partheil recommends the use of his own special form of extraction apparatus (*Zeit. angew. Chem.*, 1901, 121, 1020; *Ber.*, 1901, 34, 3611) for this purpose]. 30 c.c. of water are added, the ether driven off by warming, and the aqueous solution distilled in a current of steam until all the volatile acids have passed over. The residue, after addition of a few drops of phenolphthalein, is mixed with a slight excess of barium hydroxide. If after 15 minutes' warming on the water-bath the alkaline reaction is still persistent, a current of carbon dioxide is passed through the flask and the filtrate concentrated to 10 c.c. It is then introduced into a 150 c.c. flask, 40 c.c. of water being used for rinsing, and the liquid made up to the mark with 95% alcohol. 100 c.c. of the filtrate are evaporated on the water-bath and the residue rinsed into a beaker, acidified with hydrochloric acid and precipitated with sodium sulphate. From the weight of the barium sulphate obtained the corresponding weight of the lactic acid in 100 c.c. of wine is easily calculated.

In 23 samples of wine examined in this way, the lactic acid was found to vary between the limits 0.1185 and 0.4360 grm. per 100 c.c.

A somewhat different method for the same purpose was given by Möslinger (*Zeit. Nahr. Genussm.*, 1901, 4, 1120), but according to Trummer (*Zeit. landw. Vers.-wesen. Oester.*, 1908, 11, 492) that of Kunz gives the most accurate results. It must be noted that the

methods of both Kunz and Möslinger assume that lactic acid is not volatile with steam, which apparently is not strictly the case (see Utz, *loc. cit.*). This is a possible source of error in both methods.

*Jerusalem's Method.*—In this method (*Biochem. Zeit.*, 1908, **12**, 361 and 379) the lactic acid is estimated by oxidising it to aldehyde according to the equation  $2\text{CH}_3.\text{CH}(\text{OH}).\text{CO}_2\text{H} + \text{O}_2 = 2\text{CH}_3.\text{CHO} + 2\text{CO}_2 + 2\text{H}_2\text{O}$ . The aldehyde is estimated by treatment with an excess of iodine in alkaline solution and subsequent estimation of the amount of iodine not absorbed to form iodoform. To get trustworthy results (*vide infra*) the oxidation of the aqueous solution of the acid must be carried out by warming the liquid containing the lactic acid with sulphuric acid and adding potassium permanganate to the boiling solution. The aldehyde as it is formed must be removed as quickly as possible, and this is effected by means of a current of air. The apparatus for the absorption of the aldehyde must be very efficient, and in the original a specially devised form is described. In estimating lactic acid in animal tissues and liquids by this method, as, for example, in blood, milk and urine, the lactic acid must be first extracted with ether. Quantitative extraction is only possible when the liquid is so concentrated as to be in a pasty consistency. Details are given in the original of a special apparatus suitable for the extraction.

It is clear that this method is liable to a number of sources of error, and is useless in presence of substances which yield acetaldehyde on oxidation. It is also necessary to insure that none of the aldehyde is oxidised to acetic acid. According to von Fürth and Charnass (*Biochem. Zeitschr.*, 1910, **26**, 199) the method is untrustworthy except under very special conditions, such as the preservation of great dilution and low temperature. By maintaining certain standard conditions, which are defined in the paper referred to, a definite percentage of the theoretical yield of acetaldehyde can be obtained and the method can thus be made quantitative, but for this purpose the aldehyde should be estimated by Ripper's potassium hydrogen sulphite method (*Monatsh.*, 1900, **21**, 1079).<sup>1</sup>

*Partheil's Method.*—This method (*Zeit. Nahr. Genussm.*, 1902, **5**, 1053; see also *Ber. Deutsch. Pharm. Ges.*, 1903, **13**, 304) was devised to separate lactic acid from other volatile acids. It serves to

<sup>1</sup> See also Mondschein and von Fürth (*Biochem. Z.* **42**, 91) for use of this method in presence of  $\beta$ -hydroxybutyric acid and (*ib. p.* 105) in presence of proteins; also von Fürth and Ishihara (*Biochem. Z.* **50**, 468) for its application.

estimate lactic acid in the mixture of volatile acids obtained from wine by distillation with steam. For this purpose the mixture of acids is heated for 30 minutes with an excess of baryta water, evaporated to a small bulk and then transferred to a small distilling flask. The liquid is evaporated to complete dryness in a vacuum. After cooling, a tap funnel is fitted to the neck of the flask and the side tube connected with a nitrometer filled with potassium hydroxide solution. A few c.c. of concentrated sulphuric acid are introduced into the flask and the levels adjusted in the nitrometer. The contents of the flask are then heated carefully and the carbon monoxide formed, according to the equation  $\text{CH}_3.\text{CHOH}.\text{CO}_2\text{H} = \text{CH}_3.\text{CHO} + \text{H}_2\text{O} + \text{CO}$ , is measured. The number of cubic centimeters of carbon monoxide reduced to  $0^\circ$  and 760 mm., multiplied by 0.00402, gives the weight of lactic acid in grm. The quantity of lactic acid present in the volatile acids of wine is about 0.04%. It is, of course, essential for this method, to be trustworthy, that other substances, for example citric acid, which give rise to carbon monoxide, should be absent.

Paris (*Chem. Zent.*, 1908, [i], 773) approves of this method as giving good results, but Buchner and Meisenheimer (*loc. cit.*) state that it always gives low results. In the most favourable case they found it to be 7% low. It must be remembered, too, that Bistrzycki and Siemeradzki (*Ber.*, 1906, 39, 51) have stated that when lactic acid is heated with concentrated sulphuric acid it liberates only 80 to 85% of the theoretical quantity of carbon monoxide. On the other hand, it must be pointed out that while Buchner and Meisenheimer in condemning Partheil's method in their first communication (*Ber.*, 1908, 41, 1416) state that their own method gives nearly theoretical results, in their 1910 communication they give examples which show a deficiency of 5 to 10%. From this it would appear that there is little to choose between the two methods on the score of accuracy, whilst in most cases the method of Partheil is certainly the more rapid. Further work on the relative value of the two methods is desirable.

*Palm's Method.*—The method of Palm described on page 767 has been used as a means of estimating lactic acid and is especially applicable in a few cases. But according to Ulzer and Seidel (*Monatsh.*, 1897, 18, 138), it does not give concordant results.

### Commercial Lactic Acid

Lactic acid is largely used in dyeing and mordanting as a substitute for tartaric and citric acids, over which it possesses certain advantages. It also finds use in spirit distilleries, in tanning and for other purposes.

The following tests are made for the commercial valuation of the commercial acid (McLauchlan, *7th Intl. Congress Appl. Chem.*, 1909, 148, and *J. Amer. Leather Chem. Assn.*, 1907, 15).

(a) *Free Acid*.—20 grm. of commercial lactic acid are diluted to 250 c.c. and 25 c.c. of the dilute solution titrated direct, as rapidly as possible (cold) with N/5 sodium or potassium hydroxide, using phenolphthalein as indicator. 1 c.c. N/5 KOH = 0.0181 grm. lactic acid. The end point is taken when a pink colour appears which remains on stirring.

(b) *Anhydride*.—25 c.c. of the same dilute solution are boiled with a known excess of N/5 sodium hydroxide for 10 minutes and then, after adding sufficient N/5 acid to more than neutralise the sodium hydroxide, the excess of acid is titrated back with the alkali. The difference between the first and second estimations gives the anhydride present. This is generally from 5 to 10% of the total acidity, but may be higher.

(c) *Volatile Acids*.—These are estimated by evaporating 25 c.c. of the dilute solution to half its volume, diluting to 20 c.c., repeating the operation 2 or 3 times and finally titrating the residual material with N/5 sodium hydroxide, using phenolphthalein. The difference between results *a* and *c* gives the volatile acids. This method is not very accurate owing to the volatility of lactic acid, but it answers trade purposes.

(d) *Ash and sulphuric acid* can be estimated in the ordinary way. If free sulphuric acid or volatile acids are present, the value for the acidity in *a* does not, of course, represent lactic acid, and allowance has to be made accordingly.

The American Leather Chemists' Association has recently approved the following Provisional Method for the analysis of commercial lactic acid, which differs in some respects from the one just given (*J. Am. Leath. Chem. Assn.*, 1921, 16, 3, 137).

*Free Sulphuric Acid*.—Dissolve 50 grm. of the sample in 200 c.c. alcohol, which should be neutral, and of at least 95% strength.

Heat to  $60^{\circ}$ , cover and leave overnight in a warm place. Filter off precipitated material and wash with alcohol. Evaporate off the alcohol, make up residue to 250 c.c. with water, add 5 c.c. strong HCl, boil, add  $\text{BaCl}_2$  and determine  $\text{BaSO}_4$  in the usual way. Calculate to per cent.  $\text{H}_2\text{SO}_4$  on the original sample.

*Volatile Acids.*—Weigh out 1 grm. of sample, make up to about 50 c.c. with water, titrate with  $\text{N}/2$  NaOH. Calculate the result to lactic acid (1 c.c.  $\text{N}/2$  NaOH = 0.045 grm. lactic acid). On this basis, make up a solution containing about 15 grm. of acid per liter. Place 150 c.c. of this diluted solution in a long-necked 300 c.c. Kjeldahl flask, connected through a Kjeldahl bulb trap to a vertical spiral condenser, the total height from the bottom of the flask to the top of the turn connecting with the condenser being between 20 and 24 in. Distil over 125 c.c. in from 47 to 53 minutes, counting from the time the first drop falls into the receiver, which should be a graduated cylinder. Add 125 c.c. of water to the residue in the flask and repeat. Titrate both distillates with  $\text{N}/10$  NaOH and phenolphthalein and calculate result to grm. of acetic acid (1 c.c.  $\text{N}/10$  NaOH = 0.006 grm. acetic acid. From these figures for acid found in distillates find actual weight of volatile acid placed in boiling flask, by means of table, and calculate this result to percentage of volatile acid in the sample.

TABLE SHOWING THE RELATION OF AMOUNTS OF VOLATILE ACID FOUND IN DISTILLATE OBTAINED UNDER STANDARD CONDITIONS TO THE AMOUNTS ACTUALLY PRESENT IN DISTILLING FLASK, IN MILLIGRAMS

One Distillation							
In distillate	In flask	In distillate	In flask	In distillate	In flask	In distillate	In flask
1	0.0	14	17.5	27	37.5	40	57.9
2	0.0	15	19.0	28	39.0	41	59.6
3	0.0	16	20.5	29	40.6	42	61.3
4	2.0	17	22.1	30	42.1	43	62.9
5	3.5	18	23.6	31	43.7	44	64.6
6	5.1	19	25.2	32	45.2	45	66.3
7	6.7	20	26.7	33	46.8	46	68.0
8	8.2	21	28.2	34	48.3	47	69.8
9	9.8	22	29.8	35	49.9	48	71.5
10	11.3	23	31.3	36	51.5	49	73.3
11	12.8	24	32.9	37	53.1	50	75.0
12	14.4	25	34.4	38	54.7	51	76.8
13	15.9	26	35.9	39	56.3	52	78.5

## Two Distillations

5	0.0	22	19.2	39	38.9	56	58.6
6	1.0	23	20.4	40	40.0	57	59.8
7	2.0	24	21.5	41	41.1	58	61.1
8	3.0	25	22.7	42	42.3	59	62.3
9	4.0	26	23.9	43	43.4	60	63.5
10	5.0	27	25.0	44	44.6	61	64.7
11	6.2	28	26.2	45	45.7	62	65.9
12	7.4	29	27.3	46	46.8	63	67.2
13	8.6	30	28.5	47	48.0	64	68.4
14	9.8	31	29.7	48	49.2	65	69.6
15	11.0	32	30.8	49	50.3	66	70.8
16	12.1	33	32.0	50	51.5	67	72.0
17	13.4	34	33.1	51	52.7	68	73.3
18	14.5	35	34.3	52	53.9	69	74.5
19	15.7	36	35.4	53	55.0	70	75.7
20	16.9	37	36.6	54	56.2	71	76.9
21	18.1	38	37.7	55	57.4	72	78.1

*Free Acid and Anhydride.*—Titrate 50 c.c. of the dilution made up for volatile acid, in the cold, with  $N/2$  NaOH and phenolphthalein to first full pink. Call this figure "first titration." From it subtract the number of cubic centimeters of  $N/2$  NaOH equivalent to the sum of volatile acid and free sulphuric acid present in the 50 c.c. of dilution. (If the sample contains free oxalic or hydrochloric acid, the amount must be estimated by appropriate methods, and further deduction made.) Calculate the remainder to lactic acid and express it as a percentage of the sample. This is the free lactic acid. After completing the first titration, add 4 c.c. excess of alkali, or in the case of concentrated acids 5 c.c., and leave at room temperature ( $20^{\circ}$  to  $25^{\circ}$ ), for 15 minutes. Then add 5 c.c.  $N/2$   $H_2SO_4$ , boil, and titrate back with  $N/2$  NaOH. The amount of alkali used by anhydride is now found by subtraction and calculated to lactic acid. Express this as per cent. of lactic acid equivalent to anhydride present in sample.

The directions for the estimation of anhydride follow closely those recommended by Besson (*Collegium*, 1910, 73; *Chem. Ztg.* 1911, 35, 26), who found that the boiling method is not reliable, as the results vary greatly, according to the time of boiling, the excess of alkali used, etc.

Monin (*Rev. Gen. Mat. Col.*, 1910, 14, 279) also recognised that the boiling method gives variable results and concluded on erroneous grounds that lactic anhydride does not exist in lactic acid solutions.

The following method has been agreed upon by buyers and sellers in Germany for testing the commercial product.

*Lactic Acid Method*, 1911.—1 grm. of the acid is weighed out in a small beaker (100 to 150 c.c. capacity), diluted with 20 c.c. of water and titrated with N/1 sodium hydroxide, as free as possible from carbonate, until a permanent pink colouration is obtained. An excess of exactly 1 c.c. of alkali is then added in the case of lactic acids of medium concentration (43.5 to 50% by weight) or 3 c.c. of N/1 alkali in the case of high percentage acids, and the solution warmed on the boiling water-bath for 5 minutes. N/1 sulphuric acid is then added until the pink colour disappears and an excess of 1 c.c. of acid added; after being warmed 2 minutes on the boiling water-bath the solution is titrated with the N/1 alkali solution. The number of cubic centimeters of N/1 sodium hydroxide used multiplied by 9 gives the percentage by weight of *free acid* (that is, lactic acid +  $\frac{1}{2}$  lactic anhydride), while the total quantity of alkali used in cubic centimeters diminished by the number of cubic centimeters of N/1 acid, also multiplied by 9, gives the *total acid*.

*Example:*

1. Titration with N/1 NaOH: used 4.72 c.c. "Free-acid" = 4.72  $\times$  9 = 42.68%.

Then heated 5 minutes with an excess of 1 c.c. N/1 NaOH.

2. Titration with N/1 H<sub>2</sub>SO<sub>4</sub>: used 0.81 c.c.

Heated on water-bath 2 minutes with excess of 1 c.c. N/1 H<sub>2</sub>SO<sub>4</sub>.

3. Final titration with N/1 NaOH. Used 0.92 c.c.

*Total acid* calculation:

$$4.72 + 1.0 + 0.92 = 6.64 \text{ c.c. NaOH}$$

$$0.81 + 1.0 = 1.81 \text{ c.c. H}_2\text{SO}_4$$

$$\text{Difference} = 4.83 \text{ c.c.}$$

$$\text{Total acid} = 4.83 \times 9 = 43.47\%$$

The new method gives results lower by 0.7 to 1.5% than the old method. In the case of concentrated acids the difference may be as great as 2.5%.

Tanners usually purchase lactic acid on the basis of free acid only and maintain that the anhydride has no deliming value. But for other purposes it is purchased on the basis of total acid and anhydride.

The method of Ulzer and Seidell (*Monatsh.*, 1897, 18, 138) is sometimes used for estimating lactic acid in the commercial article.

10 grm. of lactic acid solution is diluted to 1 litre. 100 c.c. of the dilute solution is mixed with a quantity of concentrated potassium hydroxide solution representing 3 grm. potassium hydroxide, and a 5% solution of potassium permanganate gradually added, shaking constantly, until the solution, which at first is green, becomes violet. The mixture is heated to boiling, when it must remain violet; after cooling, hydrogen peroxide is added until the colour is destroyed. The solution is again boiled, filtered, the precipitate thoroughly washed and the oxalic acid formed by the oxidation is precipitated as calcium oxalate after acidifying with acetic acid. The calcium oxalate is collected, ignited and weighed as calcium oxide. It is assumed that oxidation occurs according to the equation  $2C_3H_5O_3 + 5O_2 = 2C_2H_2O_4 + 4H_2O + 2CO_2$ , so that  $CaO = C_3H_5O_3$ . The method returns lactic anhydride as lactic acid, and oxalic acid or substances yielding oxalic acid on oxidation are also counted as lactic acid.

C. F. Boehringer and Sohn of Nieder-Ingleheim a. Rh., one of the principal German manufacturers of lactic acid, point out (*Färberzeit.*, 1906, 17, 167) that as commercial lactic acid is frequently adulterated with mineral acids, the tests for sulphuric acid, hydrochloric acid, sulphates and chlorides should always be carried out. Iron should be tested for potassium ferrocyanide. The guarantee of strength should always refer to weight (grm. per 100 grm.), not to volume, as the density is greatly altered by impurities such as sugars, dextrin.

**Impurities in Commercial Lactic Acid.**—Besides water and lactic anhydride, commercial lactic acid is liable to contain the following impurities:

*Inorganic matters*, left on igniting the substance. *Sulphuric acid* and *sulphates* will be indicated on adding barium chloride to the 10% aqueous solution of the original substance; *chlorides* by silver nitrate; salts of *calcium* by ammonium oxalate; *zinc*, *lead*, and *iron* by diluting the liquid, nearly neutralising with ammonia, and introducing sulphuretted hydrogen.

*Foreign Organic Acids.*—Of these, the presence of *oxalic* or *tartaric acid* will be indicated by the formation of a precipitate on adding lime-water to alkaline reaction, and *citric acid* by precipitation occurring on boiling the liquid so obtained. *Acetic* and *butyric acids* may be recognised by their respective odours on gently heating the

liquid; or, more certainly, by the production of the fragrant odours of their respective ethyl ethers on heating the sample with alcohol and strong sulphuric acid. Ethyl lactate boils at a high temperature and has very little odour. The absence of malic acid and glycollic acid is shown (in the absence of sulphuric acid) by the 10% aqueous solution not producing a turbidity with lead acetate.

*Sarcolactic acid* may be detected by the formation of a blue *precipitate* on adding cupric sulphate to the 10% aqueous solution of the substance.

*Neutral organic matters* may, in general, be detected by the production of a brown colour on mixing the sample with an equal volume of cold concentrated sulphuric acid. *Glycerol* may be detected by treating the sample with a slight excess of zinc oxide and a little water, evaporating to dryness at 100°, and treating the residue with ether-alcohol. On evaporation of the solution, glycerol will be left as a sweet syrupy liquid. On treating the residue left undissolved by ether-alcohol with alcohol alone, *cane-sugar* and *dextrose* will be dissolved. Dextrose and other impurities will also be recognised by the formation of a red or yellow precipitate on heating the neutralised acid with Fehling's solution.

Lactic acid should not be materially coloured when heated with a strong solution of alkali hydroxide, and should be wholly soluble in ether.

**United States Pharmacopœia Requirements (9th Revision).—**Lactic acid is defined as a liquid containing lactic acid (optically inactive  $\alpha$ -hydroxypropionic acid) and lactic anhydrides equivalent to a total of not less than 85% nor more than 90% of  $C_3H_5O_2$ .

A colourless, or slightly yellow, syrupy liquid, nearly odourless, having an acid taste, and absorbing moisture on exposure to air. Miscible with water, alcohol, or ether; insoluble in chloroform, gasoline, or carbon disulphide. Strongly acid to litmus. Sp. gr. about 1.206 at 25°.

It is not vaporised at temperatures below 160°; at higher temperatures it emits inflammable vapours. Not more than 0.006 grm. of ash remains on incinerating 5 c.c. of the liquid.

On adding about 0.1 grm. of potassium permanganate to 2 c.c. of a mixture of equal parts of lactic and sulphuric acids, and heating gently, the odour of aldehyde becomes perceptible.

10 c.c. of the solution of the acid in distilled water (1:100) is not rendered opalescent by 1 c.c. of silver nitrate T.S. (chloride).

Separate portions of 10 c.c. each of an aqueous solution of the acid (1:20) remain unaffected by the addition of 1 c.c. of barium chloride T.S. (sulphate), or by 1 c.c. of copper sulphate T.S. (sarcolactic acid).

An aqueous solution of the acid does not show a precipitate of heavy metals on treatment with hydrogen sulphide.

On adding a few drops of lactic acid to 10 c.c. of hot alkaline cupric tartrate V.S., no red precipitate is produced (sugars). On adding 1 c.c. of lactic acid, drop by drop, to 5 c.c. of ether, shaking it after each addition, the ether solution does not become even transiently turbid (glycerol).

Lactic acid poured carefully upon an equal volume of colourless, concentrated sulphuric acid contained in a clean test-tube, the temperature being kept at  $15^{\circ}$ , does not give a dark-coloured zone at the line of contact upon standing for 15 minutes (organic impurities).

No turbidity is produced on heating the acid with an excess of lime water (phosphoric, tartaric, citric, or oxalic acid).

The strength of the acid is determined as follows:

Pour about 2.5 c.c. of lactic acid into a tared and stoppered 250 c.c. flask, weigh accurately, add 50 c.c. of normal potassium hydroxide V.S. and boil the liquid for 20 minutes. The residual titration of the boiling solution with normal sulphuric acid V.S., using phenolphthalein T.S. as indicator, should show not less than 85% nor more than 90% of  $C_3H_6O_3$ . (Each c.c. of N/1 potassium hydroxide V.S. used corresponds to 0.09005 grm. of lactic acid or anhydride calculated as  $C_3H_6O_3$ .)

**British Pharmacopœia Requirements (1914).**—Lactic acid is defined as an aqueous solution containing not less than 75% by weight of hydrogen lactate ( $HC_3H_6O_3$ ) and not less than 10% by weight of lactide ( $C_3H_6O_4$ ). It may be obtained by the fermentation of lactose. The sp. gr. is about 1.21. The solution has to satisfy the following tests: Each grm. diluted with 10 c.c. of water should require for neutralisation not less than 8.3 c.c. of N/1 sodium hydroxide solution; after the further addition of 10 c.c. of the alkaline solution, not more than 8.6 c.c. of N/1 sulphuric acid should be required to neutralise the excess of alkali. It should yield no characteristic reactions for copper, iron, chlorides, citrates, oxalates, phosphates, sulphates, or tartrates. Lead limit 10 parts per million; arsenic limit 5 parts per million.

The acid when diluted gives no precipitate with solution of copper sulphate (absence of *sarcolactic acid*) and none, or only the slightest trace, with excess of solution of potassio-cupric-tartrate, even after prolonged boiling (absence of more than traces of various sugars). The mixture obtained by heating lactic acid with excess of zinc carbonate and evaporating to dryness should not, when exhausted with absolute alcohol, and the latter evaporated, yield any sweet residue (absence of *glycerol*). Gently warmed there should be no rancid odour (absence of *fatty acids*). Carefully poured upon an equal volume of sulphuric acid contained in a test-tube little or no darkening should ensue (absence of *organic impurity*). No turbidity, either permanent or transient, should be produced when the acid is added, drop by drop, to twice its volume of ether (absence of *gum, sugar, mannitol, calcium phosphate*). It should give no precipitate with a solution of lead subacetate (absence of *malic and sulphuric acids*).

**Salts of Lactic Acid.**—*Metallic Lactates* are all more or less soluble in water, but usually dissolve only sparingly in the cold. They are all insoluble in ether. The *sarcolactates* are usually more soluble than the salts of ordinary lactic acid and are lævorotatory.

*Calcium Lactate*,  $\text{Ca}(\text{C}_3\text{H}_5\text{O}_3)_2 \cdot 5\text{H}_2\text{O}$ , is obtained in crystals when lactic acid is neutralised with lime or chalk, and the liquid concentrated. It crystallises in small white mammillated tufts, which under the microscope appear as delicate rhombic needles, some of which look like bundles bound in the centre. From acid solutions, a so-called *acid lactate* of calcium (a compound of calcium lactate with lactic acid) crystallises in radiating trimetric needles or fibrous masses. One part of calcium lactate dissolves in 9.5 parts of cold water, and in all proportions in boiling water or alcohol (compare calcium sarcolactate, page 786). When calcium lactate is heated, it readily parts with its water of crystallisation, and at  $250^\circ$  to  $260^\circ$  is converted into a tumefied mass containing calcium dilactylate,  $\text{O}[\text{CHMe.CO}_2]_2\text{Ca}$ , from which absolute alcohol dissolves out any unaltered lactate, leaving the dilactylate as a sparingly soluble residue. The corresponding dilactylic acid melts at  $106^\circ$  (Jungfleisch and Godchot, *Compt. Rend.*, 1907, 144, 979).

*Ferrous Lactate*,  $\text{Fe}(\text{C}_3\text{H}_5\text{O}_3)_2 \cdot 3\text{H}_2\text{O}$ , crystallises in light yellow needles, soluble in 48 parts of cold or 12 parts of boiling water. The dry salt is permanent, but the solution rapidly oxidises. Ferrous

lactate is not infrequently adulterated, the substances used for the purpose being dried ferrous sulphate, milk-sugar, and starch. A sample of "lactate of iron," examined by M. Peltier, contained 25% of ferrous sulphate and 75% of milk-sugar. *Ferrous sulphate* can be readily detected by the copious precipitate produced on treating the solution of the sample with barium chloride. *Milk-sugar* may be detected by rendering the solution alkaline with soda, passing hydrogen sulphide to precipitate the iron, filtering, adding Fehling's solution to the filtrate, filtering rapidly in the cold from the copper sulphide, and heating the filtrate, when a yellow or red precipitate of cuprous oxide will be formed if milk-sugar be present. *Starch* may be detected in the portion of the sample insoluble in cold water by the blue colour produced on addition of solution of iodine.

*Lead Lactate*,  $\text{Pb}(\text{C}_3\text{H}_5\text{O}_3)_2$ , is freely soluble in water, sparingly soluble in cold, but readily in hot alcohol, and slightly soluble in ether. It does not crystallise, but forms a gummy mass on evaporation. (*Glycerate* of lead is but slightly soluble in cold water.) By adding lead acetate and alcoholic ammonia, lactic acid is completely precipitated as a compound containing  $3\text{PbO}, 2\text{C}_3\text{H}_5\text{O}_3$  (compare page 767).

*Zinc Lactate*,  $\text{Zn}(\text{C}_3\text{H}_5\text{O}_3)_2, 3\text{H}_2\text{O}$ , crystallises from concentrated solutions in shining crusts, or from dilute solutions in four-sided prismatic needles, soluble in 53 parts of water at  $15^\circ$ , or 6 of boiling water, and insoluble in alcohol (compare zinc sarcosylactate, page 786). The water of crystallisation is lost rapidly at  $100^\circ$ , and above  $210^\circ$  the salt decomposes.

*Magnesium Lactate*,  $\text{Mg}(\text{C}_3\text{H}_5\text{O}_3)_2, 3\text{H}_2\text{O}$ , forms a white crystalline powder or prismatic needles and is soluble in 23.8 parts of water at the ordinary temperature. It is used to some extent in pharmacy. *Bismuth Lactate* also finds use in medicine.

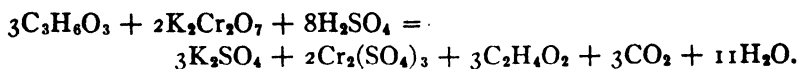
"*Lactolin*," which has found considerable application in wool dyeing, especially as an assistant to the dichromate mordant, is *acid potassium lactate*, or an equimolecular mixture of lactic acid and normal potassium lactate (Kielmayer, *Färberzeit.*, 1899, **10**, 17 and 33; Dreher, *ibid.*, 68).

"*Antimonin*" is calcium antimony lactate (Düring, *Färb. Zeit.*, 1900, **11**, 319. Chem. Werke Schuster und Wilhelmy, D. R. P. 216158). *Sodium antimony lactate* and *sodium calcium antimony lactate* have been patented by von Heyden (D. R. P. 184202). They

are used as tannin-fixing mordants. Compounds of tannic acid with lactic acid are used in the leather industry (Dreher, Eng. patents, 9556, 1901; 22629, 1901; 23188, 1901; 9556, 1902).

*Glycerol mono- and di-lactates* (Kalle and Co., D. R. P. 21697, 1910) are employed therapeutically as lactic acid substitutes.

**Estimation of Lactic Acid in Lactates.**—For this purpose Paessler's method (*Collegium*, 1907, 388 and 396) is generally used. It gives good results in some cases when other methods are either useless or too laborious. It depends on the oxidation of lactic acid to acetic acid and carbon dioxide in sulphuric acid solution by a standard solution of potassium dichromate solution according to the equation



An accurately weighed portion of the material (about 0.4 grm.) or an aliquot proportion of a suitably diluted solution, is boiled with 10 c.c. of 10% sulphuric acid and 25 c.c. of N/2 potassium dichromate solution, in an Erlenmeyer flask fitted with a reflux condenser. The excess of dichromate solution remaining is then titrated in the ordinary way with N/10 sodium thiosulphate solution after adding 10 c.c. of 10% potassium iodide solution. Starch paste is used as final indicator in the usual manner. 1 c.c. of N/2 potassium dichromate = 0.01127 grm. of lactic acid. Volatile matter which reduces chromate solution should be removed by repeated evaporation before the estimation. Sugar, dextrin, glycerol or similar materials which reduce chromate solution and cannot be removed by volatilisation render the method useless. Good results are obtained by this method with such salts as calcium antimony lactate, and sodium antimony lactate, containing more or less excess of lactic acid. In such cases the antimony is first precipitated as antimony sulphide by hydrogen sulphide, the solution filtered and any excess of hydrogen sulphide removed by boiling. Lactic anhydride is not oxidised by the above treatment and, if present, must first be converted into lactate by heating with a slight excess of alkali.

**Active Lactic Acids.**—By adopting the principle previously applied by Pasteur to the preparation of dextrorotatory and lævorotatory tartaric acids from inactive racemic acid, T. Purdie (*Jour. Chem. Soc.*, 61, 754) found that ordinary inactive lactic acid can be decomposed into two oppositely active lactic acids by fractional crystallisa-

tion of the strychnine salt, that of the lævorotatory acid being the less soluble. The two free acids exhibit equal and opposite optical activities to those of their salts. The zinc salts of each of the optically active acids crystallise with  $2\text{H}_2\text{O}$ . On mixing aqueous solutions of equal quantities of the two zinc salts, and stirring the liquid, inactive zinc lactate containing  $3\text{H}_2\text{O}$  separates. The two optically active modifications of lactic acid present a very close resemblance to ordinary lactic acid and to each other. The anhydride and salts of dextrolactic acid are lævorotatory, whilst the anhydride and salts of lævolactic acid are dextrorotatory. On heating, both active modifications yield the same lactide, and when this takes up the elements of water, it yields ordinary inactive lactic acid. Lævolactic acid was stated by Lewkowitsch (*Ber.*, 1883, **16**, 2720) to be produced by growing the mould *Penicillium glaucum* in a solution of ammonium lactate. A repetition of this experiment by Linossier (*Bull. Soc. Chem.*, [3], **6**, 10) gave an opposite result, the residual lactic acid yielding lævorotatory salts. F. Schardinger (*Monatsh.*, **11**, 551) obtained lævolactic acid by the fermentation of cane-sugar by the *Bacillus acidi lævolactici*, which closely resembles the ordinary lactic acid bacillus in appearance, but has considerably greater fermentative power.

**Dextrolactic Acid. Sarcolactic Acid. Paralactic Acid.**—This acid is obtainable in the manner above described from inactive lactic acid. It occurs naturally in the juices of muscular tissue, in bile, and in the urine of persons poisoned by phosphorus. It is also obtained in numerous impure fermentations (*e. g.*, of dextrin, dextrose, cane-sugar, milk-sugar, etc.). Frankland and Macgregor obtained sarcolactic acid by the interrupted bacterial fermentation of ordinary inactive calcium lactate (*Jour. Chem. Soc.*, 1893, **63**, 1028).

It is generally present in organs undergoing pathological change. During muscular activity the proportion of lactic acid present is increased.

Sarcolactic acid presents the closest resemblance to ordinary lactic acid. The most tangible distinctions are: (1) The dextrorotation of free sarcolactic acid, and the lævorotation of its anhydride and salts; ordinary lactic acid, when pure, both in the free state and in the form of salts, being optically inactive. Fermentation lactic acid frequently contains more or less of the active forms. (2) The solu-

bility and amount of water of crystallisation in the calcium and zinc salts. (3) Ordinary lactic acid yields a deep blue liquid on addition of cupric sulphate, whilst sarcolactic acid is almost completely precipitated by that reagent.

Sarcolactic acid is conveniently prepared by dissolving extract of meat in 4 parts of water, and adding to the solution 3 times its volume of methylated spirit. The filtered liquid is evaporated to a syrup, which is again treated with alcohol, filtered, evaporated, acidified with sulphuric acid, and extracted with ether. The acid thus obtained on evaporation of the ether may be purified by conversion into the zinc salt.

When pure, *d*-lactic acid forms flattened, radiating prisms, melts at  $25^{\circ}$  to  $26^{\circ}$  and is very hygroscopic. In 10.5% aqueous solution  $[\alpha]_D^{15} = +3.82$ ; in 2.5% solution  $[\alpha]_D^{15} = +2.67$ .

According to Yoshikawa (*Z. physiol. Chem.*, **87**, 382), the optical rotation of solutions of lithium *d*-lactate varies directly as the concentration. The *d*-lactic acid is separated from the mixture under investigation as the zinc salt, and this is boiled for 30 minutes with  $\text{Li}_2\text{CO}_3$  to convert it to the lithium salt. If  $\alpha$  is the observed rotation, the concentration will be equal to

$$\frac{(0.2864 - \sqrt{0.082025 - 0.0162\alpha})}{0.0081}$$

On heating, sarcolactic acid yields a laevorotatory anhydride, the solution of which is laevorotatory; but this active anhydride and the lactide formed on further heating are hydrolysed by water to a solution of ordinary inactive lactic acid.

*Calcium sarcolactate*,  $\text{Ca}(\text{C}_3\text{H}_5\text{O}_3)_2 + 4\text{H}_2\text{O}$ , is soluble in about 12 parts of cold water. The solution is laevorotatory, the value of  $[\alpha]_D$  for a solution containing 5.35% of the anhydrous salt being stated by Wislicenus to be  $-5.48^{\circ}$ .

*Zinc sarcolactate*,  $\text{Zn}(\text{C}_3\text{H}_5\text{O}_3)_2 + 2\text{H}_2\text{O}$ , crystallises in slender needles which lose their water slowly at  $100^{\circ}$ , and give off empyreumatic vapours below  $150^{\circ}$ . The salt is soluble in 17.5 parts of water at  $15^{\circ}$ , but only sparingly soluble in cold alcohol (distinction from ethylene-lactic acid). The aqueous solution of zinc sarcolactate is laevorotatory, the following being the values for  $[\alpha]_D$  found by Wislicenus for solutions of various strengths:

$c = 16.05^1$	$[\alpha]_D = -6.36$
11.01 <sup>1</sup>	- 6.36
7.47	- 6.83
6.13	- 7.41
5.26	- 7.60

<sup>1</sup>Supersaturated.

**Lævolactic Acid.**—This as stated above was first obtained by Schardinger. Friedländer's pneumonia bacillus produces *l*-lactic acid from a number of carbohydrates, disaccharides and glycerol.

*l*-Lactic acid melts at 26° to 27°. Its salts have the same composition and solubility as the salts of *d*-lactic acid; they are, however, dextro-rotatory, the dextrorotation being equal in magnitude, under the same conditions, to the lævorotation of the *d*-salts.

**Lactic Acid in Organic Tissues and Extracts.**—The detection and estimation of lactic acid in organic tissues, organs, etc., can only be carried out after removing fats and proteins. The material is extracted with water and albumin removed from the extract by coagulation, by boiling after adding a little dilute sulphuric acid. Afterwards the liquid is accurately neutralised with baryta at the boiling point, and, after filtering, the liquid is evaporated to a syrup. This is precipitated with absolute alcohol and the precipitate thoroughly extracted with alcohol. From the united alcohol extracts, the alcohol is distilled off, and the neutral residue shaken with ether to remove fats. The residue is then taken up with water, and after addition of phosphoric acid, again shaken with ether, which now removes the lactic acid. From the united ethereal extracts (the extraction is best carried out as described under the Buchner and Meisenheimer method, page 771), the ether is distilled off, the residue is dissolved in water and carefully warmed on the water-bath to remove ether and volatile acids. The filtered solution is then boiled with zinc carbonate to obtain a solution of zinc lactate which is evaporated until crystallisation begins; 15 c.c. of alcohol is added and the solution is then left in a desiccator over sulphuric acid, and treated as in Buchner and Meisenheimer's method. The resulting crystalline salt should be analysed (water and zinc oxide) to make certain of its identity, and the optical rotation observed, so as to distinguish between the different forms of lactic acid likely to occur.

**Ethylene Lactic Acid. Hydracrylic Acid.**— $\beta$ -Hydroxypropionic Acid.



This compound is distinguished from the other modifications of lactic acid by the fact that it yields no trace of lactide when heated, being resolved, almost without residue, into water and acrylic acid,  $\text{CH}_2:\text{CH}.\text{CO}_2\text{H}$ . The same decomposition occurs on heating it with sulphuric acid diluted with an equal weight of water. On the other hand, when acrylic acid is heated to  $100^\circ$  with excess of sodium hydroxide solution, hydracrylic acid is reproduced. Hydracrylic acid has only been obtained by synthetical means,<sup>1</sup> its formation from  $\beta$ -iodopropionic acid by boiling with water or heating with moist silver oxide being the most available reaction.

On oxidation with nitric acid, hydracrylic acid yields carbon dioxide and oxalic acid. With chromic acid mixture, the former is the sole product of the action.

The sodium and calcium salts of hydracrylic acid melt without change at about  $140^\circ$  to  $145^\circ$ , but at a higher temperature they lose water and are converted into acrylates. Zinc hydracrylate,  $(\text{C}_3\text{H}_3\text{O}_3)_2\cdot\text{ZN}, 4\text{H}_2\text{O}$ , crystallises in large shining prisms, soluble in an equal weight of cold water (100 parts of salt dissolve in 89 parts of water at  $16.5^\circ$ ). The salt is also soluble in alcohol, which precipitates zinc lactate and sarcosylactate from their aqueous solutions.

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<sup>1</sup> Wislicenus obtained from flesh, together with sarcosylactic acid, an acid, which he supposed to be ethylene-lactic acid, but it was shown by Siegfried (*Ber.*, 1889, 22, 2713) that this was really acetyl-lactic acid.

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